Is cAMP Good or Bad?

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Is cAMP good or bad? Depends on where it's made.

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Endothelium of the vascular system forms a semipermeable barrier between blood and the interstitial space that serves to control and restrict the luminal to abluminal movement of water, plasma proteins and other solutes.[1] During inflammation, mediators such as thrombin, histamine and platelet activating factor (PAF) induce vascular leakage defined as an increased endothelial permeability to plasma proteins. In the lung, disruption of the barrier formed by pulmonary microvascular endothelial cells (PMVECs) occurs during inflammatory disease states such as acute lung injury and acute respiratory distress syndrome. Endothelial permeability to macromolecules occurs via the formation of small gaps between (paracellular) or through (transcellular) cells, and is controlled by cell shape and cell adhesion through a balance of opposite mechanical forces: contractile forces generated by actomyosin motor function; tethering forces generated by adhesive proteins at the cell-cell border and focal adhesions at the cell–matrix border.[2] Due to its central role in mechanical processes, Ca^{2+} is an important regulator of endothelial permeability. Intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) is increased in PMVECs upon binding of proinflammatory mediators to their respective membrane receptors, and subsequent activation of the G_q protein-mediated signaling cascade. In particular, this rise in $[Ca^{2+}]_i$ is essential for the generation of endothelial cell paracellular gaps. Other downstream major actors in this Ca^{2+}-sensitizing cascade include PKC, Ca^{2+}-dependent myosin light chain kinase (MLCK) and the monomeric GTPase RhoA.

Whereas elevated $[Ca^{2+}]_i$ increases endothelial barrier permeability, increased cAMP has the opposite effect.[3] Changes in this ubiquitous second messenger is governed by modulating the cAMP synthesis and cAMP hydrolysis machinery. In endothelial cells, most of cAMP synthesis is accounted for by the Ca^{2+}-inhibited type 6 adenylyl cyclase (AC6),[4] and most of cAMP hydrolysis is due to two isoforms of cyclic nucleotide phosphodiesterases (PDEs), PDE3 and

PDE4.[5,6] AC6 is located at the plasma membrane and is activated by several stimulatory G protein (G_s)-coupled receptors (G_sPCRs), such as adenosine A2, prostaglandin E_1 (PGE_1), or β_2-adrenergic (β_2-AR) receptors. Elevation of cAMP due to activation of these receptors, direct G_s activation with cholera toxin, direct AC6 activation by forskolin, or application of membrane permeant cAMP analogues all appear to increase cell-cell and cell-matrix tethering, decrease isometric tension development, decrease intercellular gap formation, and decrease permeability in multiple experimental preparations.[3,7] Of particular importance is the observation that elevation of cAMP counteracts the hyperpermeability of PMVECs evoked by inflammatory mediators, providing a possible mechanism for the anti-edema effect of β_2-adrenergic agonists.[8] Most of the mechanisms by which cAMP regulates endothelial permeability involve activation of cAMP-dependent protein kinase (PKA) and phosphorylation of PKA substrate proteins, such as MLCK,[9] ERK1/2[10] and RhoA.[11] However, recent evidence suggest that cAMP can also act in a PKA-independent manner, through its direct binding to Epac, a guanine nucleotide exchange factor for the small GTPase Rap1.[12-14]

The trigger of an inflammatory process causing endothelial permeability dysfunction is a pathogenic insult. Many pathogenic bacteria secrete toxins to alter the intracellular concentration of cAMP.[15] Some of these toxins (e.g. cholera and pertussis toxins) disrupt the normal AC regulation in the host cell through ADP-ribosylation of the host G_s and G_i proteins, thereby activating endogenous AC and elevating intracellular cAMP.[7] Other bacterial toxins catalyze themselves the synthesis of cAMP in the host cell: this is the case of the invasive AC of *Bordetella pertussis*, the edema factor of *Bacillus anthracis* (the etiological agent of anthrax), the AC of *Yersinia pestis*, and ExoY of *Pseudomonas aeruginosa*.[15] Surprisingly, while cholera toxin protects endothelial cell barrier function,[7,16] some of the other AC toxins have been reported

to induce endothelial permeability. This is the case of ExoY which has been reported by Sayner *et al.*[17] to induce PMVEC gap formation while increasing intracellular cAMP concentration up to 800-fold. Genetically modified *Pseudomonas aeruginosa* to introduce a catalytically deficient ExoY strain did not increase cAMP and did not increase PMVEC permeability.[17] Why then is cAMP protective on endothelial barrier when synthesized by endogenous AC and deleterious when produced by ExoY? Sayner *et al.*[17] propose an explanation by demonstrating that ExoY localizes exclusively to the cytosol, while endogenous AC activity is located at the plasma membrane. Moreover, they found that rolipram, a selective PDE4 inhibitor, increased the concentration of cAMP generated by endogenous AC, but not that produced by ExoY.[17] Thus, ExoY and AC6 determine two different pools of cAMP, and cAMP in each pool produces opposite outcomes on endothelial cell function.

This hypothesis was further tested in a study performed by the same group which appears in this issue of *Circulation Research*.[18] In this study, Sayner *et al.* used rat PMVECs infected with an adenovirus encoding an engineered soluble AC (sAC) made of a chimeric construct of portions of the cytosolic domains of mammalian type I and type II enzymes (sACI/II).[19,20] Unlike the bicarbonate-sensitive human sAC expressed in testis which is insensitive to forskolin,[21] sACI/II is forskolin-sensitive.[19] Also, unlike ExoY which confers a strong basal AC activity to PMVEC host cells, cells expressing sACI/II construct show no basal AC activity. These two features allowed the authors to evaluate the respective contribution of endogenous membrane AC6 vs. exogenous sACI/II in controlling barrier permeability. They show that upon forskolin application, cAMP increases exclusively in the plasma membrane fraction in control or GFP-infected PMVECs, but increases in both membrane and cytosolic fraction in sACI/II-infected cells. Activation of β-adrenergic receptor or PDE4 inhibition specifically affects the

membrane cAMP pool but leaves the cytosolic pool unchanged. The most striking result of their study is that forskolin reduces the permeability in control or GFP-infected PMVECs, but increases permeability by the formation of endothelial gaps in sACI/II-infected cells.[18] Control experiments show that this phenomenon is absent when sACI/II has been engineered to relocate to the plasma membrane.

The two studies by Sayner *et al.*[17,18] provide clear evidence for a functional significance of cAMP compartmentation in PMVECs as well as a variation on a theme of the seminal discovery made in cardiac tissue in the late 1970's by Brunton and colleagues.[22] Experiments in isolated cardiac myocytes have confirmed the paradigm that cAMP is unevenly distributed within the cell.[23,24] In particular, different maneuvers to activate cAMP synthesis, e.g. forskolin vs. β-adrenergic stimulation of AC[23,25] or heterologous expression of the non-cardiac Ca^{2+}-stimulated AC8 in cardiac myocytes,[26,27] have shown that specific pools of cAMP can control the activity of different proteins. A molecular mechanism that supports such a phenomena is that localized activation of PKA occurs at discrete sites within the cell because the kinase and other cAMP effectors are localized through their interaction with A-Kinase Anchoring Proteins (AKAPs).[28] Of particular interest is the recent findings that these cAMP compartments are controlled by the activity of specific PDE isoforms.[23] In particular, a PDE4 subtype (PDE4D3) was shown recently to form complexes with mAKAP (a muscle-specific AKAP[29]) respectively at the nuclear[30] and SR membrane,[31] controlling local cAMP/PKA signalling. Similarly, another PDE4 subtype (PDE4D5) forms a complex with β-arrestin, a protein which controls desensitization of β_2-adrenergic receptors,[32] and a PDE3 subtype (PDE3B) forms a complex at the cardiac sarcolemmal membrane with the G protein-coupled, receptor-activated phosphoinositide 3-kinase γ (PI3Kγ).[33] Such local cAMP signalling complexes must contribute to maintaining a normal

cellular function, since disruption of such complexes can lead to cellular hypertrophy[30] and heart failure.[31,33]

Based on the above studies in cardiac myocytes, follow-up studies on the work of Sayner *et al.*[17] will have to explore the molecular architecture underlining the functional cAMP compartments in PMVECs. For instance, do anchored PDEs determine the fate of cAMP generated at the membrane upon AC activation? Sayner *et al.*[17] showed that PDE4 controls the membrane pool of cAMP but not the soluble pool. One would then expect that PDE inhibitors would disrupt local membrane cAMP signalling, allow cAMP to homogenously distribute within the cells, hence transforming a protective effect of cAMP on endothelial cell barrier permeability into a destructive one. However, such hypothesis is not supported by earlier studies which indicate that different PDE inhibitors decrease microvascular permeability in a similar manner as G_sPCR agonists.[6,34] Another direction worth pursuing would be to examine the role of another, cAMP closely related cyclic nucleotide, cGMP. Although the role of cGMP in controlling microvascular permeability is much less consensual than that of cAMP, there has been a number of reports indicating that cGMP raises the endothelial cell barrier permeability.[35,36] Some of the complexity in the action of cGMP may arise from the complex crosstalks that exist between cGMP and cAMP signalling cascades.[37] But an interesting feature of cGMP signalling directly related to the work of Sayner *et al.*[17] is that cGMP is 'normally' synthesized both at the membrane and in the cytosol. Using natriuretic peptides (ANP, BNP, CNP) to activate the particular guanylyl cyclase (GC)[38] or nitric oxide to activate the soluble GC[39] provides a unique means to separately manipulate membrane and cytosolic compartments, and explore the functional outcomes on endothelial cell barrier permeability.

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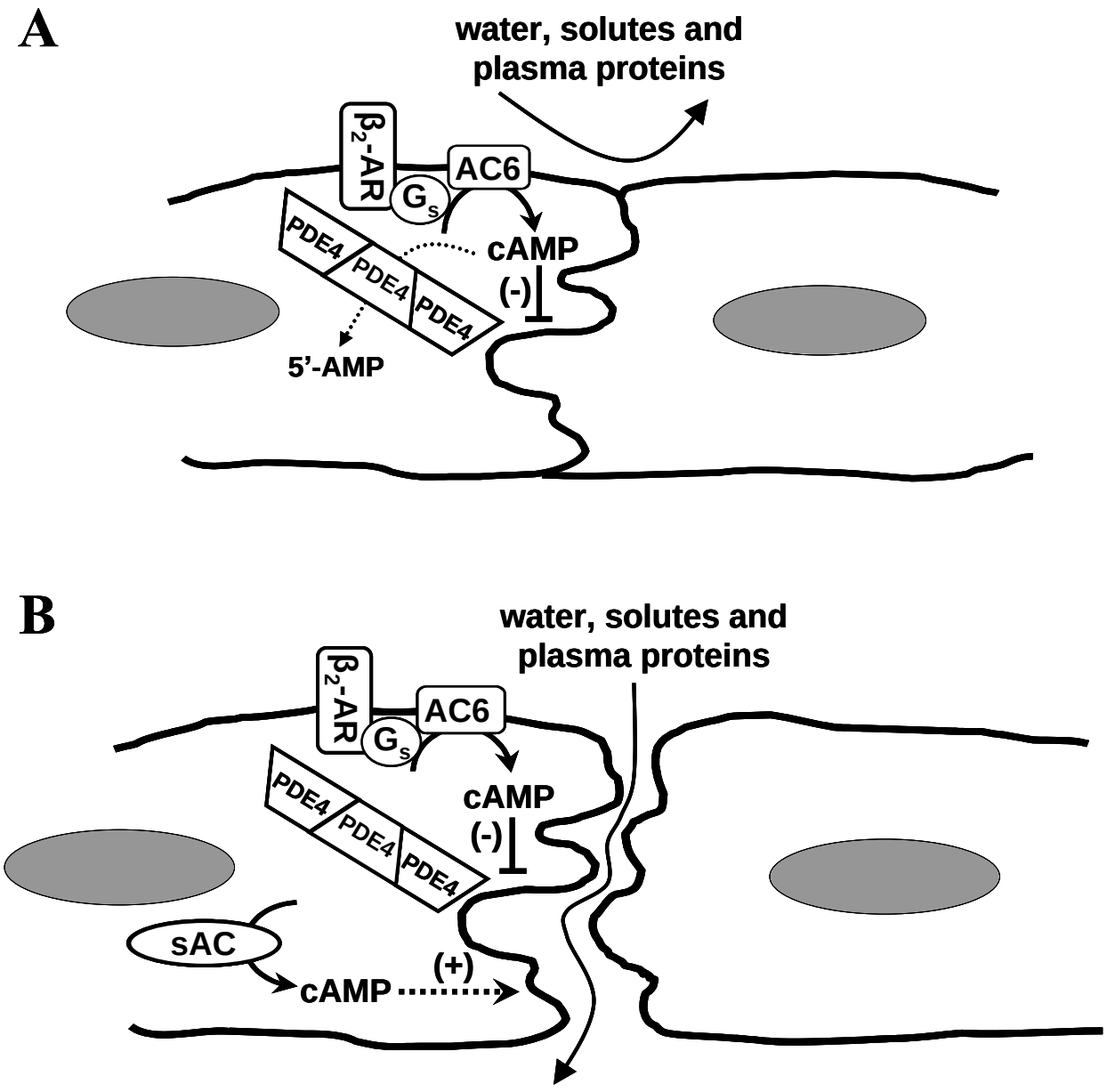
Figure Legend

cAMP compartments in pulmonary microvascular endothelial cells. A, Upon activation of a G_s-coupled receptor (e.g. β_2-AR), endogenous plasma membrane adenylyl cyclase (AC6) synthesizes cAMP in a membrane compartment delimited by type 4 phosphodiesterase (PDE4) activity. By inhibiting formation of paracellular gaps, membrane cAMP improves pulmonary microvascular endothelial barrier and prevents the luminal to abluminal movement of water, plasma proteins and other solutes. B, Soluble adenylyl cyclases (sAC), like ExoY and sACI/II, localize outside this membrane domain, away from AC6, PDE4, and membrane receptors. cAMP synthesized in a cytosolic pool overwhelms plasma membrane cAMP and disrupts the endothelial barrier. Exactly where these soluble cyclases reside and what intracellular cAMP effectors mediate their barrier disruptive actions is not known.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