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Soluble adenylyl cyclase in health and disease ☆

Andreas Schmid [a], Dimirela Meili [a,b], Matthias Salathe [a,*]

[a] Division of Pulmonary, Allergy, Critical Care and Sleep Medicine, University of Miami School of Medicine, 1600 NW 10th Ave, RMSB #7058 (R-47), Miami, FL 33136, USA
[b] Department of Internal Medicine, University Hospital Zürich, Rämistrasse 100, 8091 Zurich, Switzerland

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ABSTRACT

The second messenger cAMP is integral for many physiological processes. Soluble adenylyl cyclase (sAC) was recently identified as a widely expressed intracellular source of cAMP in mammalian cells. sAC is evolutionary, structurally, and biochemically distinct from the G-protein-responsive transmembranous adenylyl cyclases (tmAC). The structure of the catalytic unit of sAC is similar to tmAC, but sAC does not contain transmembranous domains, allowing localizations independent of the membranous compartment. sAC activity is stimulated by HCO_3^-, Ca^{2+} and is sensitive to physiologically relevant ATP fluctuations. sAC functions as a physiological sensor for carbon dioxide and bicarbonate, and therefore indirectly for pH. Here we review the physiological role of sAC in different human tissues with a major focus on the lung. This article is part of a Special Issue entitled: The role of soluble adenylyl cyclase in health and disease, guest edited by J. Buck and L.R. Levin.

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1. Introduction

The second messenger cyclic adenosine monophosphate (cAMP) was discovered in 1958 [1,2]. Since then, it is recognized as one of the most important and evolutionarily conserved second messengers for many signaling pathways [3]. cAMP can activate three main effector proteins: cyclic-nucleotide-gated (CNG) ion channels [4], guanine-nucleotide exchange proteins activated by cAMP (Epac) [5] and cAMP-dependent protein kinase (PKA) [6,7].

cAMP is generated from ATP by adenylyl cyclases (ACs). There are six different classes of ACs distributed throughout bacteria, archaea and eukarya. These classes are unrelated in sequence and structure, but all produce cAMP [8]. All eukaryotic adenylyl cyclases belong to class III [3]. Vertebrate animals were felt to have only one family of hormone and G-protein regulated enzymes with a transmembranous component (tmAC). In mammals, this family contains nine tmACs transcribed from 9 different genes, which differ in their tissue and developmental expression as well as in their regulatory properties [3,9]. In 1999, Levin and Buck cloned a genetically unrelated AC in rat testis, guided by a cyclase activity originally described in the 1970s that was different from tmAC [10,11]. The activity was originally described by Braun in 1975 as a Mn^{2+} responsive AC in rat testis [12]. At discovery, the enzyme was named "soluble Adenylyl Cyclase" (sAC) as it was found in the cytosolic compartment of rat testis preparations [12]. Later, it was shown that most of sAC was not soluble in the cytoplasm, but found in discrete locations such as the nucleus, mitochondria, centrioles or cilia [13–15]. Fig. 1 summarizes the activation mechanisms if different adenylyl cylcases.

2. Structure of sAC

Mammalian nucleotidyl cyclases contain two fairly well preserved catalytic domains. These two domains (C_1 and C_2), by associating with each other, form the catalytic core. The C_1C_2 heterodimers shape into two sites at the interface: the active site and a degenerated, inactive pocket [16,17]. sAC and tmACs are monomeric proteins and catalyze cAMP production through dimerization of their two catalytic domains [18]. They share homology of the two catalytic domains, but sAC lacks 2 hydrophobic domains, each representing 6 membrane-spanning helices that localize tmAC to membranes [19].

Recently, the crystal structure of the catalytic domains of sAC was described [17,20]. The human catalytic units reveal a secondary structure similar to the one from cyanobacteria but differences are seen in some external loops. The cyanobacterial sAC has two fully identical nucleotide binding sites. In contrast, only one of these sites is accessible for ATP in the human form [20]. Interestingly, there is a sequence of 3 consecutive proline residues between C_1 and C_2 (220–222) locally related to a hydrophobic patch [17]. These structures have been described as potential protein binding sites [21]. They offer the possibility of an interaction area for proteins or other sAC domains [17] which could allow dimerization of sAC splicing forms that only contain one catalytic unit.

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* Corresponding author. Tel.: +41 305 243 6388; fax: +41 305 243 6992.
E-mail address: msalathe@med.miami.edu (M. Salathe).

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In contrast to other mammalians as the dog or some anthropoids, humans have a single sAC gene [22]. By alternative splicing, several sAC isoforms are generated [15,23,24] and an additional alternative start site has recently been described, indicating a considerable isoform diversity [25]. Full-length sAC (sAC_{fl}) includes an N-terminus with the two catalytic domains (~1100 amino acids spanning 33 exons). Exclusion of exon 12 generates a truncated isoform, sAC_t (amino acids 1–490), which contains just the two sAC catalytic domains [17]. Although the half maximal stimulation for HCO_3^- and the K_m for Mg^{2+} and Mn^{2+} and Ca^{2+} are the same for sAC_t and sAC_{fl}, the truncated form is 10 times more active than sAC_{fl} [10,26]. This is explained by an autoinhibitory domain in the C-terminal tail of sAC_{fl} [27] that is not present in the truncated form. Splice variants of the sAC_{fl} found in testis and skeletal muscle also contain a heme-binding domain that could bind NO or CO. A detailed description of alternative splicing in bronchial epithelial cells will be given below.

3. Cell compartmentalization and microdomains

cAMP is a second messenger that can signal at different locations in a single cell [7]. For this purpose, a tight spatial and temporal control of the cAMP concentration is critical. The cell has two strategies to do this. First, cAMP production and utilization are spatially confined. Whereas tmACs are restricted to membranes, sAC can be localized throughout the cell and is found in mitochondria, nuclei, centrioles, the mitotic spindle [13] as well as cilia [15,28]. At these locations, ACs are anchored together with PKA [29,30] by scaffold proteins such as A-kinase anchoring proteins (AKAP), allowing local utilization. Second, the diffusion of cAMP is confined by the degradation of cAMP by PDEs [31] and the apical actin web, which requires functioning cystic fibrosis transmembrane conductance regulator (CFTR) in some epithelial cell types [32]. This fence mechanism together with the local production and utilization of cAMP creates "microdomains" of cAMP signaling.

Microdomains of cAMP signaling may not be captured by whole cell cAMP measurements. Better suited are measurements in intact cells using reporter protein constructs (e.g., PKA or Epac) that allow recordings in subcellular compartments [33], for instance, using fluorescence resonance energy transfer (FRET).

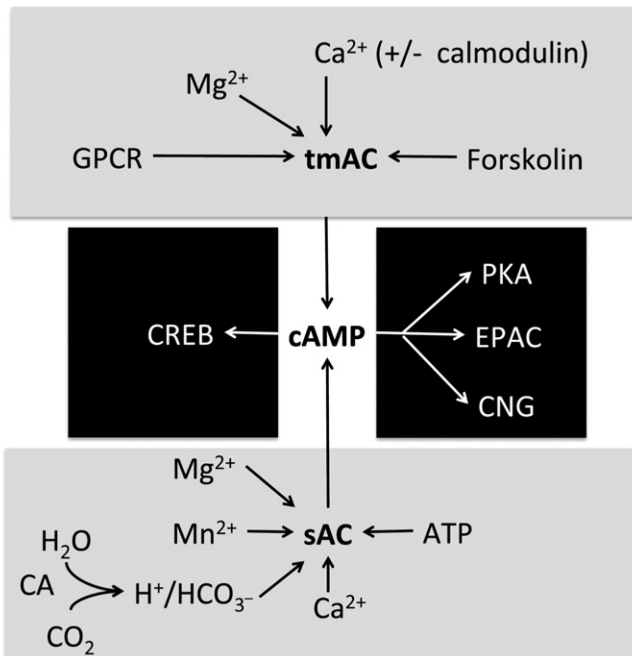

Fig. 1. Regulation of cAMP production. Upper Panel: cAMP is produced by tmAC, which can be stimulated by G-protein coupled receptors (GPCR). tmACs are also stimulated by forskolin, a diterpene that is not activating sAC. tmACs require bivalent ions such as Mg^{2+} and Ca^{2+} for the cyclization of cAMP from ATP. Calcium signals activate some of the 9 known tmAC over calmodulin whereas calmodulin is not required for signaling in some other tmACs. Lower Panel: sAC is stimulated by HCO_3^- which is in an equilibrium with H_2O and CO_2 catalyzed by carbonic anhydrase (CA). sAC also requires calcium (independent of calmodulin) for cAMP production and is stimulated by magnesium. Magnesium can be substituted by manganese. Middle Panel: cAMP signals over different mechanism in the body: Protein kinase A (PKA), the guanine-nucleotide exchange proteins activated by cAMP (Epac) and the cyclic-nucleotide-gated (CNG) ion channels. In the nuclear compartment cAMP can affect gene expression over cAMP response element-binding protein (CREB).

4. Regulation of sAC

The product of both sAC and tmAC is cAMP, but the regulation of the two enzymes is completely different. G-protein-coupled receptors and heterotrimeric G proteins [34] regulate tmAC, allowing a broad spectrum of signaling input. These regulation mechanisms do not apply to sAC. Forskolin, a plant diterpene that binds to the degenerated pocket of the catalytic unit of tmACs [17], triggering a strong enzymatic response, does not affect sAC signaling. An inserted loop in the three dimensional configuration of sAC's catalytic domains tightens the available space in the degenerated pocket, preventing forskolin binding [17].

sAC is directly activated by HCO_3^-, leading to increased substrate turnover and reduced substrate inhibition and by Ca^{2+}, enhancing substrate binding [35]. In this way, small changes in the intracellular concentration of bicarbonate and calcium ions may significantly affect local cAMP levels [36]. Finally, sAC is sensitive to variations in intracellular ATP concentrations [37]. These regulation mechanisms have been preserved throughout evolution from cyanobacteria to humans [17], supporting the importance of sAC in cell signaling.

4.1. Calcium

Calcium plays a role in regulating sAC and certain tmACs. At physiological concentrations, Ca^{2+} stimulates the tmACs AC1, AC3 and AC8 isoforms via calmodulin and inhibits AC5 and AC6 by displacing the cofactor Mg^{2+} [36,38]. In contrast, mammalian sAC is stimulated by Ca^{2+} in a calmodulin independent way [3,26,35] by lowering the K_m for Mg^{2+} ATP [20]. Like other class III adenylyl cyclases, sAC requires two divalent cations for activity. It is active if Mg^{2+} is the only available divalent ion, but addition of Ca^{2+} increases the affinity for its substrate ATP to values consistent with the concentration of ATP found inside the cells [35]. This suggests that in vivo, mammalian sAC utilizes both Mg^{2+} and Ca^{2+}, and that its activity will be sensitive to ATP fluctuations inside the cells. Modeling of sAC activity suggests that Ca^{2+} bound to the γ-phosphate of ATP enters the catalytic site where it interacts with specific residues in the sAC catalytic center, resulting in an "open sAC state". Mg^{2+}, the second divalent metal then binds to the α-phosphate of ATP, leading to a "closed state". The change from "open" to "closed" states induces the release of the β- and γ-phosphates and esterification of the remaining α-phosphate with C3 of the ribose in adenosine ("cyclizing") [19].

4.2. Bicarbonate

HCO_3^- stimulates substrate turnover via an allosteric change of sAC leading to closure of the active site, recruitment of the catalytic Mg^{2+}, and rearrangement of the phosphates in the bound ATP as it has been shown in the sAC homolog of cyanobacterial adenylyl cyclase (CyaC). This facilitates cAMP formation and release [19].

Through its HCO_3^- regulation, sAC has been shown to function as a physiological CO_2/HCO_3^- (and thus indirectly pH) sensor in many tissues [39]. The sAC-dependent mechanisms for sensing HCO_3^- in extracellular fluids are similar in shark gills, kidney and epididymis using either HCO_3^- movement through channels or diffusion of CO_2 through the membrane [40,41]. This becomes particularly effective when sAC

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