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Review Article

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Roles of Soluble Adenylyl Cyclase in cAMP Synthesis in Animal Cells

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Abstract

The second messenger cyclic adenosine 3′,5′-monophosphate (cAMP) synthesis is catalyzed by adenylyl cyclases (ACs). The second messenger cAMP participates in many physiological processes in cells. All eukaryotic adenylyl cyclases (transmembrane adenylyl cyclase and soluble adenylyl cyclases) belong to class III. Soluble adenylyl cyclase was identified in many studies as a widely expressed intracellular source of cAMP in mammalian and non-mammalian cells. Soluble adenylyl cyclase is evolutionary, structurally, and biochemically distinct from the G-protein-responsive transmembrane adenylyl cyclase. sAC is distributed throughout the cytoplasm, and it may be present in the nucleus and in mitochondria. sAC has been confirmed to be a bicarbonate sensor in a variety of mammalian cell types. Here I review the physiological role of soluble adenylyl cyclase in different mammalian and non-mammalian tissues. These data promote further research to clarify the exact roles of soluble adenylyl cyclase in the development of the biological activity of normal cells in the body and the therapeutic implications.

KEYWORDS
Adenylyl cyclase; Transmembrane adenylyl cyclase; Soluble adenylyl cyclase; Forskolin; cAMP.

INTRODUCTION

Cyclic adenosine-3′,5′-monophosphate (cAMP) is a cytosolic second messenger formed from ATP through the action of adenylyl cyclases and/or its degradation by phosphodiesterases (PDEs). It was discovered by Earl Sutherland during his studies of hormonal regulation of metabolism in mammalian heart and liver (Berthet *et al.*, 1957; Sutherland and Rall, 1958). There are six different classes of adenylyl cyclases distributed throughout Bacteria, Archaea and Eukaryota; these classes are unrelated in sequence and structure but all produce cAMP as a result of convergent evolution (Linder and Schultz, 2008). In mammals, cAMP is formed by either of two types of widely expressed Class III ACs, including nine transmembrane enzymes (tmACs) and one soluble AC (sAC) (Kamenetsky *et al.*, 2006). tmACs reside in the cell membrane and they are directly regulated by heterotrimeric G proteins and generate cAMP in response to hormones (Nguyen *et al.*, 2018) and neurotransmitters, which signal through G protein-coupled receptors (Taussig and Gilman, 1995). sAC is insensitive to forskolin and heterotrimeric G protein regulation (Buck *et al.*, 1999) and it is only regulated by calcium (Jaiswal and Conti, 2003; Litvin *et al.*, 2003) and bicarbonate (Chen *et al.*, 2000). sAC is widely expressed (Sinclair *et al.*, 2000) and is present at discrete sub-cellular localizations in a wide variety of cells (Zippin *et al.*, 2004).

STRUCTURE AND MECHANISM OF SOLUBLE ADENYLYL CYCLASES

Mammalian sAC is composed of two heterologous catalytic domains (C1 and C2) in the N-terminus (~ 50 kDa). Many putative regulatory domains such as an auto inhibitory region (Chaloupka *et al.*, 2006), a canonical P-loop and leucine zipper sequences (Buck *et al.*, 1999) are found at the C terminus (~ 140 kDa). The C1C2 heterodimers shape two sites at the interface: the active site and a degenerated, inactive pocket (Kleinboelting *et al.*, 2014; Sinha and Sprang, 2006). cAMP production is catalyzed by the dimerization of the two catalytic domains of the sAC and tmACs monomeric proteins (Kamenetsky *et al.*, 2006). 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 (Steegborn *et al.*, 2005). Full-length sAC (sACfl) includes an N-terminus with the two catalytic domains (~ 1,100 amino acids spanning 33 exons). Exclusion of exon 12 generates a truncated isoform, sACt (amino acids 1 - 490), which contains just the two sAC catalytic domains (Kleinboelting *et al.*, 2014) and the truncated form is 10 times more active than sACfl (Buck *et al.*, 1999). sACt, sACfl and other splice variants are stimulated by HCO_3^- and divalent cations, i.e. Mg^{2+}, Mn^{2+} and Ca^{2+} (Chaloupka *et al.*, 2006; Geng *et al.*, 2005).

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Moreover, most tmACs are potently activated by forskolin, a plant diterpene that has been classically used to study cAMP function *in vivo* (Seamon and Daly, 1981; Seamon *et al.*, 1981). However, sAC is insensitive to forskolin (Buck *et al.*, 1999). Mammalian sAC and tmACs also display differential sensitivity to the pharmacological antagonists KH7 and derivatives of catechol estrogen (selective for sAC), and P-site inhibitors such as 2′,5′-dideoxyadenosine and 2′,3′-dideoxyadenosine (selective for tmAC) (Tresguerres *et al.*, 2011).

ROLE OF SAC IN TESTIS AND SPERM CELLS

In mammalian

In 1975, soluble adenylyl cyclase (sAC) is described for the first time in the cytosol of rat seminiferous tubules and epididymal sperm by Braun and Dods, distinct from the transmembrane adenylyl cyclase (tmAC), which could be stimulated by Mn^{2+} and potentiated by calcium (Braun, 1975; Braun and Dods, 1975). The presence of sAC allows cAMP to be produced in defined subcellular compartments, where it serves local signaling functions (Buck *et al.*, 1999). The catalytic active portions of sAC (C1 and C2) are conserved in cyanobacteria and myxobacteria, suggesting an evolutionary continuity between the bacterial and mammalian sAC-cAMP signaling systems.

Involvement of sAC in sperm function was confirmed by genetic ablation of the sAC gene: sAC null sperm are morphologically normal but immotile (Esposito *et al.*, 2004). Multiple studies have shown that sperm capacitation is a HCO_3^- and Ca^{2+}-dependent process (Boatman and Robbins, 1991; Gadella and Harrison, 2000; Lee and Storey, 1986; Neill and Olds-Clarke, 1987; Shi and Roldan, 1995; Visconti *et al.*, 1995). The first connection between HCO_3^-, Ca^{2+}, and cAMP metabolism was demonstrated by the Garbers group (Garbers *et al.*, 1982).

They showed that the presence of HCO_3^- and Ca^{2+} extracellular increase intracellular cAMP within 1 minute in guinea pig spermatozoa. When either HCO_3^- or Ca^{2+} was removed from the medium, only a slight increase of cAMP was observed. Okamura *et al.* (1985) reported that HCO_3^- directly activates adenylyl cyclase activity (Okamura and Sugita, 1983) and consequently increases cAMP levels in boar sperm (Okamura *et al.*, 1985), and was able to induce spermatozoa motility. Similar results were also reported in bovine and hamster spermatozoa (Garty *et al.*, 1988; Visconti *et al.*, 1995).

Independently of these reports, other studies have described the presence of a soluble adenylyl cyclase in testicular extracts (Kornblihtt *et al.*, 1981). Analogous to sperm adenylyl cyclase, the soluble testicular enzyme did not appear to be responsive to G proteins and was more active in the presence of Mn^{2+} than in the presence of Mg^{2+} (Kornblihtt *et al.*, 1981). Surprisingly, the sAC catalytic domain presents homology to cyanobacterial adenylyl cyclase which is also HCO_3^--dependent (Chen *et al.*, 2000; Wang *et al.*, 2007).

As mentioned, cAMP levels peak 1 minute after sperm exposure to HCO_3– (Battistone *et al.*, 2013; Brenker *et al.*, 2012) and then return to basal levels over the incubation period. In addition

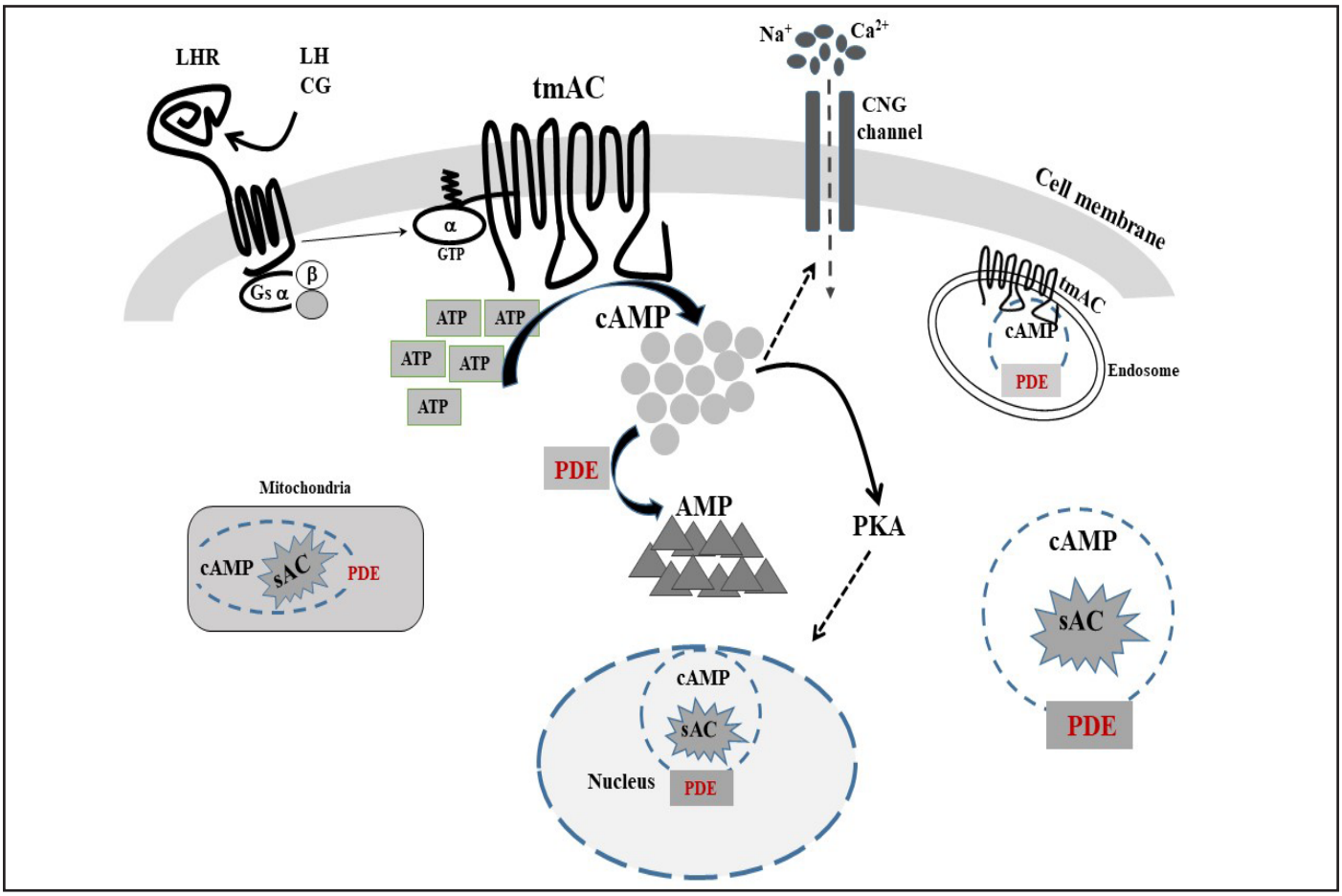

Fig. 1. Intracellular synthesis of cyclic adenosine-3',5'-monophosphate (cAMP). cAMP synthesis occurs in discrete sub-cellular localizations such as in the membrane, the cytoplasm, the mitochondria, or the nucleus. cAMP is formed by soluble adenylyl cyclase (sAC) or transmembrane adenylyl cyclase (tmAC); the phosphodiesterases (PDE) degrade cAMP formation and high levels of cytosolic cAMP lead to the activation of protein kinase A (PKA). tmAC in the cell membrane requires modulation by G-protein-coupled receptors and heterotrimeric G-protein. sAC is present throughout the cytoplasm and in organelles such as the mitochondria, the nucleus, the endosome, and other microdomains.

to responding to HCO_3^-, sAC is also a Ca^{2+} sensor. In the presence of Ca^{2+}, the KM for Mg^{2+} -ATP is reduced to levels that are close to the ATP content found in cells, turning sAC into a signal transducer highly sensitive to physiologically changes of HCO_3– (Zippin *et al*., 2013). sAC is thus presumed to be involved in spermatozoa motility, but it needs to be obliterated either genetically or pharmacologically to demonstrate its role.

When the sAC gene was eliminated using homologous recombination, the phenotype was found sterile (Esposito *et al*., 2004; Hess *et al*., 2005). The spermatozoa in which this gene was knocked out were immotile and did not undergo the cAMP/PKA-dependent increase in tyrosine phosphorylation linked to the capacitation process (Hess *et al*., 2005). When they were incubated with HCO_3^- in the capacitation-supporting media, basal levels of cAMP in sAC-/- sperm were so low that they could not be measured (Esposito *et al*., 2004).

KH7 is a competitive inhibitor with ATP-Mg^{2+} and blocked recombinant sAC activity with an IC50 of 10 μM (Han *et al*., 2005). KH7 was shown to block cAMP production in sperm, the increase in tyrosine phosphorylation, and spermatozoa motility (Hess *et al*., 2005). Altogether, these results are consistent with the hypothesis that sAC mediates the HCO_3^--induced increase in cAMP necessary to initiate capacitation. Moreover, in the absence of sAC, sperm have reduced ATP levels, do not undergo hyperactivation and fail to fertilize in vitro (Hess *et al*., 2005; Xie *et al*., 2006).

Spermatozoa need sAC for normal motility, and the capacitation process induces sAC-dependent cAMP elevation that promotes the phosphorylation of protein tyrosine. Capacitation also induces a change in motility to a hyperactivated pattern, but the sAC-dependent increases in cAMP and the phosphorylation of protein tyrosine is not required for this change. The cAMP required for acrosomal exocytosis seems to be provided by tmAC and not by sAC (Hess *et al*., 2005).

In non-mammalian

The first reports about sACs are from the dogfish shark and not from mammalian (purified recombinant protein) (Tresguerres *et al*., 2010) and from purple sea urchin (semi-purified native protein) (Beltran *et al*., 2007; Nomura and Vacquier, 2006; Nomura *et al*., 2005). The first AC protein (190 kDa) was identified from animal spermatozoa, was not stimulated by forskolin or by G-proteins, and showed high activity in response to Mn^{2+}. However, as mammalian sAC would not be discovered until almost a decade later, the sea urchin AC was not identified as a sea urchin sAC and its sensitivity to HCO_3^- was not tested either at the time. Eventually, analysis of the sea urchin sAC sequence indicated it was a homolog of mammalian sAC (Nomura *et al*., 2005). Compared with mammalian sAC, sea urchin sAC contains multiple amino acid insertions (16-74 amino acids in length) with several potential phosphorylation sites (Nomura *et al*., 2005). Dogfish shark sAC is ~ 110 kDa (Tresguerres *et al*., 2010), but it is still unclear whether sAC splice variants or isoforms exist in dogfish shark.

Like mammalian sAC, dogfish shark and sea urchin sAC are stimulated by HCO_3^-, potently stimulated by millimolar concentrations of Mn^{2+}, and inhibited by micromolar concentrations of Ca^{2+}, KH7 and derivatives of catechol estrogens. Sea urchin sAC displays a steep sensitivity to pH between 7.0 and 7.5 (Beltran *et al*., 2007), unlike the pH-insensitive mammalian (Chen *et al*., 2000) and dogfish shark sAC (Tresguerres *et al*., 2010).

Sea urchin sAC concentrates in the proximal half of the sperm flagellum near the mitochondrial midpiece (Bookbinder *et al*., 1990), suggesting that sea urchin sAC triggers the initiation of sperm motility, which depends on cAMP-dependent phosphorylation of flagella-associated proteins (Bracho *et al*., 1998). Sea urchin sAC is tightly complexed with several proteins of the plasma membrane and the axoneme, including dynein heavy chains 7 and 9, sperm-specific Na^+/H^+ exchanger, cyclic nucleotide-gated ion channel, sperm-specific creatine kinase, membrane-bound guanylyl cyclase, cGMP-specific phosphodiesterase 5A, egg peptide speract receptor, and α- and β-tubulins (Nomura and Vacquier, 2006). The authors suggested that this complex modulates sperm motility in response to speract and pH changes. Further research using confocal microscopy revealed that sea urchin sAC is also present in the head and acrosomal area (Beltran *et al*., 2007). Indeed, sea urchin sAC is important, but not essential for sperm acrosome reaction. Searches of expressed sequence tag and transcriptome shotgun assemblies databases reveal that sea urchin sAC mRNA is also present in embryonic primary mesenchyme cells (Zhu *et al*., 2001), suggesting various physiological roles in addition to sperm motility and acrosome reaction.

cAMP production in molluscs is entirely dependent on the activation of tmAC. However, the publications on cAMP and its physiological responses predates the discovery of sAC in mammals. For example, cAMP production in some bivalve species such as Mytilus galloprovincialis and Tapes philippinarum, as well as the sea hare, Aplysia californica, which did not react with/or were only slightly stimulated by forskolin (Valbonesi *et al*., 2004). Although the results of these studies have been explained by attributing activity to orthologs related to the forskolin-insensitive tmAC 9 isoform, orthologs of sAC can provide an equally plausible alternative explanation.

Two isoforms of sAC in the genome of the coral Acropora digitifera have also been identified (Barott *et al*., 2013). cAMP production in homogenates is significantly stimulated by HCO_3– and the HCO_3^--stimulated activity is inhibited by KH7 (Barott *et al*., 2013). The biological role of sAC in corals is still unknown. Because [HCO_3^-] in coral tissues ranges from ~ 4 mmol l−1 in the dark to over 100 mmol l−1 in the light (Furla *et al*., 2000), coral sAC is likely to be sensitive to physiologically relevant variations in [HCO_3^-] and most active in the light. Indeed, endogenous cAMP levels in corals in vivo are highest in the light (Barott *et al*., 2013), but it remains to be determined whether this is due to sAC activity.

sAC orthologs have also been found from other animals, including species from the placenta (*Trichoplax adns*), sponges (*Amphimedon queenslandica*), hornworm (*Saccoglossus kowalevskii*), amphioxus (*Branchiostoma floridae*), sea squid (*Ciona gutis*), orca whale (*Orcinus orca*), bottlenose dolphin (*Tursiups truncatus*), manatee (*Trichechus manatus*) and walrus (*Odobenus rosmarus*) (Tresguerres *et al*., 2014).

sAC genes are also present in the sequenced genomes or whole genome shotgun databases of appendicularia (*Oikopleura dioica*), green sea turtle (*Chelonia mydas*), painted turtle (*Chrysemys picta*), American alligator (*Alligator mississippiensis*), snake (*Python morulus*) (Wang *et al*., 2001), salmon (*Salmo salar*), chimera (Callorhinchus milii), little skate (Leucoraja erinacea), coelacanth (*Latimeria chalumnae*) and spotted gar (*Lepiosteus oculatus*), and in transcriptome shotgun assemblies of rainbow trout (*Oncorhynchus mykiss*) (Tresguerres *et al*., 2014).

In addition, the sAC protein was detected in gill, rectal gland, white muscle, intestine and eye of leopard shark (Triakis semifasciata) and round ray (*Urobatis hallerii*) by western blotting using antibodies against dogfish shark sAC (Roa and Tresguerres, 2017). sAC has been found in the testis of dogfish shark and rainbow trout, suggesting that sAC is important for sperm biology in fishes as it is in mammals.

ROLE OF sAC IN OTHER CELLS

Leydig

Recently, there are proposed roles for sAC in Leydig cells function. sAC was immuno-detected in the cytoplasm of mLTC-1 Leydig cells. The four different sAC inhibitors (KH7, LRE1, 2-CE and 4-CE) were found to inhibit LH-stimulated cAMP accumulation and progesterone level in mLTC-1 and testosterone level in mouse testicular Leydig cells. The strong synergistic effect of HCO_3– under LH stimulation further supports the involvement of sAC in the response to LH (Nguyen *et al*., 2021).

Pancreas

In mammals, glucose homeostasis is regulated by pancreatic β cells. When serum glucose is elevated, it triggers the release of insulin by β cells, which in turn makes muscle, liver, and fat to store glucose. It is well known that an increased external glucose concentration stimulates cAMP production while modulating the release of insulin (Charles *et al*., 1973). The source of this cAMP, however, was still unknown until a study using INS-1E insulinoma cells showed that glucose and glucagon-like peptide-1 (GLP-1) produce cAMP with distinct kinetics via different adenylyl cyclases (Rutter, 2001). GLP-1 induces a quick cAMP signal mediated by G protein-responsive tmACs, whereas glucose leads to a slow cAMP increase mediated by HCO_3^-, Ca^{2+}, and ATP-sensitive sAC (Ramos *et al*., 2008).

Kidney

Several sAC splice variants are presumed to be present in the kidney (Chen *et al*., 2000; Geng *et al*., 2005; Hallows *et al*., 2009; Pastor-Soler *et al*., 2003). sAC are preferentially expressed in cells of the medullary and cortical thick ascending loop of Henle, in cells of the distal tubule and in cells of the collecting duct (Hallows *et al*., 2009; Pastor-Soler *et al*., 2003). Because of sAC presence throughout the nephron, it leads to integrate external (tubular fluid) and internal (plasma, renal interstitium) signals with appropriate responses through cAMP signaling.

sAC was identified as a sensor that detects luminal bicarbonate and activates the vacuolar proton-pumping ATPase (V-ATPase) via cAMP to regulate tubular pH. sAC could regulate acid-base balance (Brown *et al*., 2012).

sAC is also involved in blood pressure homeostasis since specific sAC inhibition by KH7 causes reduced Na^+ reabsorption (Hallows *et al*., 2009). Inhibition of sAC by KH7 has been recently shown to significantly reduce CREB-mediated promoter activity. Moreover, KH7 and anti-sAC siRNA significantly decrease mRNA and protein levels of the α subunit of ENaC and Na^+/K^+ -ATP. sAC inhibition causes significant endothelial cell softening. This suggests that sAC is a regulator of gene expression involved in aldosterone signaling and an important regulator of endothelial stiffness (Schmitz *et al*., 2014).

Lung

sAC was found in airway epithelial cells. sAC is expressed in ciliated cells from human bronchial epithelia and regulates ciliary beat frequency (CBF) (Schmid *et al*., 2007). HCO_3–modulates CBF in a pH-independent way through production of cAMP by sAC in cells of healthy lung donors (Schmid *et al*., 2010; Schmid *et al*., 2007). HCO_3– related increase in CBF can be blocked by sAC inhibitors KH7 and 2-CE as well as by PKA inhibition, but not through inhibition of tmAC (Schmid *et al*., 2010; Schmid *et al*., 2007). The C2 sAC KO mice also lost their sAC-dependent CBF control (Chen *et al*., 2014).

sAC and bicarbonate transporters have been described in the arterial and venous pulmonary circulation, but a bicarbonate cAMP response was only found in the venous endothelium (Obiako *et al*., 2013). cAMP produced by tmAC in the endothelium was shown to tighten the endothelial barrier, whereas sAC activation weakened it (Obiako *et al*., 2013). sAC is expressed in pulmonary microvascular endothelial cells and pulmonary artery endothelial cells. Adding extracellular bicarbonate decreases resistance across the pulmonary microvascular endothelial cells monolayer and increases the filtration coefficient in the isolated perfused lung above osmolality controls.

Nervous system

sAC expression was found in the carotid body and in peripheral chemoreceptors (Nunes *et al*., 2009). However, in 2013, Nunes *et al*. showed that sAC does not have a physiological role in the cAMP production in isohydric hypercapnia in the carotid body. This is because sAC expression is lower than tmAC expression, and that the changes of cAMP are not dependent on different HCO_3^- and CO_2 concentrations or influenced by KH7 (Nunes *et al*., 2013).

Actually, some splice variants of sAC (Choi *et al*., 2012) are involved in a mechanism of metabolic coupling between neurons and astrocytes. sAC was highly expressed in astrocytes. sAC activity by HCO_3^- increased intracellular cAMP, which leads to glycogen breakdown and the delivery of lactate to neurons for use as an energy substrate (Choi *et al*., 2012).

sAC is also present in developing neurons, where, depending on the origin of the neuron, it is located in cell bodies, dendrites, axons and/or growth cones (Wu *et al*., 2006). The effects of sAC overexpression, i.e. axonal outgrowth and elaboration of growth cones, have similarities with morphological changes caused by the treatment of axons with Netrin1. In cultured dorsal root ganglia and spinal commissural neurons, sAC inhibition by KH7, catechol estrogens or siRNA, blocked netrin-1-induced growth cone elaboration and axonal growth (Wu *et al*., 2006). Using pharmacological and siRNA approaches, it was found that sAC activity is required for netrin-1-induced cAMP generation leading to netrin-1–mediated growth cone elaboration and axon outgrowth (Wu *et al*., 2006).

Prostate cells

A significant overexpression of soluble adenylyl cyclase (sAC) was found in human prostate carcinoma cell lines LNCaP and PC3. Suppression of sAC activity by treatment with the sAC-specific inhibitor KH7 or by sAC-specific knockdown mediated by siRNA or shRNA transfection prevented the proliferation of prostate carcinoma cells, led to lactate dehydrogenase release, and induced apoptosis. Cell cycle analysis revealed a significant rise in the G2 phase population 12 hours after sAC inhibition, which was accompanied by the down-regulation of cyclin B1 and cyclin-dependent kinase 1 (CDK1). sAC-dependent regulation of proliferation involves the EPAC/Rap1/B-Raf signaling pathway (Flacke *et al*., 2013).

Cardiovascular system

sAC was also shown to play several functions in the cardiovascular system. It has been shown to mediate apoptosis in

several models, including simulated ischemia/acidosis in coronary endothelial cells (Kumar *et al*., 2009) and cardiomyocytes (Appukuttan *et al*., 2012) and oxysterol- and oxidative stress-induced apoptosis in vascular smooth muscle cells (Appukuttan *et al*., 2013; Kumar *et al*., 2014). In fact, sAC-generated cAMP activates PKA to phosphorylate the proapoptotic Bcl-2-family member Bax. This causes the translocation of Bax to the mitochondria; interestingly, sAC was also found to translocate to the mitochondria. After reperfusion, the mitochondrial pathway of apoptosis is activated, with stereotypical radical oxygen species production, cytochrome-c release, and caspase-9/-3 cleavage. Pharmacological or genetic inhibition of sAC during ischemia, but not during the reperfusion phase of injury, suppressed these features of apoptosis (Kumar *et al*., 2009).

Eye

sAC was confirmed to be present in primary cultures of bovine corneal endothelial cells, and sAC activation increased Cystic fibrosis transmembrane conductance regulator (CFTR) dependent secretion of Cl^-, HCO_3^- and/or ATP (Sun *et al*., 2003). Although these studies were performed prior to the availability of sAC inhibitors, all data suggest that cAMP produced by sAC stimulates PKA phosphorylation of apical CFTR, thus increasing apical Cl^- permeability (Dunn *et al*., 2006; Sun *et al*., 2003). It was also demonstrated that higher HCO_3^- in culture media increased sAC expression in corneal endothelial cells (Sun *et al*., 2004).

A role of sAC in retinal ganglion cells (RGCs) was also investigated. Retinal cells express HCO_3^- transporters and carbonic anhydrases (Adamus and Karren, 2009; Casey *et al*., 2009). Inhibition of sAC activity in RGCs using 2-CE (Hallows *et al*., 2009) or anti-sAC shRNA decreased RGC survival, while HCO_3– (Sun *et al*., 2004) increased survival and axon growth in RGCs (Corredor *et al*., 2012). The elimination of sAC in early retinal development impacted RGC development and influenced amacrine and photoreceptor differentiation as shown by differences in cell numbers and layer thickness, respectively (Shaw *et al*., 2016). Another study showed that relative levels of phosphorylated CREB and phosphorylated Bcl-2 were decreased in corneal endothelial cells treated with 2-CE or sAC siRNA, suggesting that HCO_3^--dependent endogenous sAC activity can mobilize anti-apoptotic signal transduction (Li *et al*., 2011).

CONCLUSION

cAMP is synthesized by transmembrane adenylyl cyclase and/or soluble adenylyl cyclase. Since cAMP can be found in many intracellular locations, it might actually depend on sAC even though it was previously thought to depend on tmACs. What identifies sAC and makes it possible to distinguish it from other ACs is its signature obtained by stimulating it with HCO_3^- and/or divalent cations like Mg^{2+}, Mn^{2+} or Ca^{2+}. This review shows that sAC is present in many tissues and suggests that it is in charge of major physiological roles.

CONFLICT OF INTEREST

The author declare that no conflict of interest exists.

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