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Bicarbonate, carbon dioxide and pH sensing via mammalian bicarbonate-regulated soluble adenylyl cyclase

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Soluble adenylyl cyclase (sAC; ADCY10) is a bicarbonate (HCO_3^-)-regulated enzyme responsible for the generation of cyclic adenosine monophosphate (cAMP). sAC is distributed throughout the cell and within organelles and, as such, plays a role in numerous cellular signalling pathways. Carbonic anhydrases (CAs) nearly instantaneously equilibrate HCO_3^-, protons and carbon dioxide (CO_2); because of the ubiquitous presence of CAs within cells, HCO_3^--regulated sAC can respond to changes in any of these factors. Thus, sAC can function as a physiological HCO_3^-/CO_2/pH sensor. Here, we outline examples where we have shown that sAC responds to changes in HCO_3^-, CO_2 or pH to regulate diverse physiological functions.

1. Introduction to cyclic adenosine monophosphate signalling

Since its initial discovery nearly 70 years ago, the second messenger cyclic adenosine monophosphate (cAMP) has been established as a key player in various biological processes such as development, proliferation and apoptosis [1]. Often, cAMP plays multiple roles within a single cell; to prevent unintended interactions between different cellular pathways, cAMP signalling is compartmentalized into distinct microdomains which control the temporal and spatial limits of individual cAMP signalling cascades [2]. Contained within these microdomains are essential components of cAMP signalling, including the enzymes which generate the second messenger, its effectors, and the enzymes which degrade cAMP. The family of enzymes responsible for producing cAMP from adenosine triphosphate (ATP) are adenylyl cyclases (ACs) [3,4]. Downstream targets of cAMP include protein kinase A (PKA), exchange protein activated by cAMP (EPAC) [5,6] and cyclic nucleotide-regulated channels [7]. These effectors are found within close proximity to the enzymes that synthesize and degrade cAMP; e.g. via PKA-tethering A-kinase anchoring proteins (AKAPs) [8]. Finally, phosphodiesterases (PDEs) degrade cAMP to control its diffusion and are responsible for defining the boundaries of individual microdomains [9–12] (figure 1). Ultimately, the compartmentalization of cAMP signalling pathways to microdomains enables this ubiquitous second messenger to simultaneously facilitate multiple, and oftentimes opposing, biological processes throughout a cell.

In mammalian cells, the 10 known AC isoforms can be divided into two classes: the G-protein-regulated transmembrane ACs (tmACs: ADCY1–9) and the HCO_3^--regulated soluble AC (sAC:ADCY10) [13]. In most tissues, AC activity is detected predominantly, if not exclusively, in particulate fractions from cells; thus, cAMP was thought to be produced exclusively by membrane-bound proteins [14,15]. Molecular cloning of nine mammalian tmAC genes (*ADCY1–9*) revealed the presence of multiple transmembrane domains providing molecular confirmation that tmACs are membrane-bound proteins [16,17]. Biochemical characterization of tmACs confirmed that they are regulated by G-proteins and mediate the cellular responses to hormones and neurotransmitters signalling via G-protein-coupled receptors (GPCRs) [17]. However, cAMP microdomains

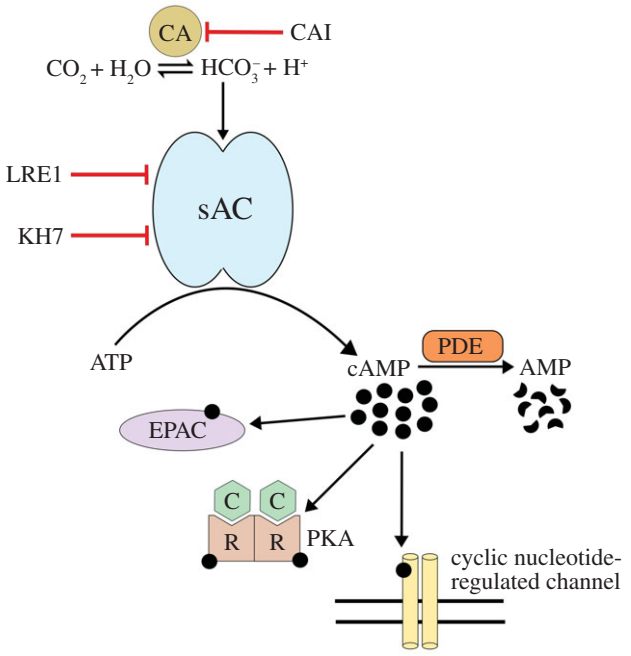

Figure 1. cAMP signalling mediated by the HCO_3^-/CO_2/pH-regulated soluble adenylyl cyclase (sAC). Inside of a cell, carbonic anhydrases (CAs) rapidly interconvert CO_2 and water into HCO_3^- and protons. HCO_3^- activates sAC, and, due to the nearly instantaneous equilibrium between HCO_3^-, CO_2 and pH, sAC activity can fluctuate in concert with changes to any of these factors. cAMP produced by sAC will bind to and activate a downstream effector protein (protein kinase A, PKA; exchange proteins activated by cAMP, EPAC; and/or cyclic nucleotide-regulated channels) and be degraded by phosphodiesterases (PDEs) into AMP. LRE1 and KH7 are sAC-specific inhibitors and CAI represents carbonic anhydrase inhibitors.

do not solely exist at the plasma membrane; they can be found throughout the cytoplasm or within several distinct cellular compartments [4,18,19]. sAC regulates cAMP inside these intracellular microdomains [4,19–22].

2. History of soluble adenylyl cyclase

In the mid-1970s, Theodor Braun detected a novel 'soluble' AC activity in cytosolic extracts from mammalian testis [23]. Unlike the previously identified tmACs, soluble AC activity was not associated with the plasma membrane, and it was insensitive to stimulation by G-proteins or the plant-derived pharmacological tmAC activator forskolin (FSK) [24,25]. A related activity was detected in spermatozoa, and this activity was stimulated by bicarbonate (HCO_3^-) [26–29]. The molecular source of soluble AC activity remained elusive until, in 1999, our laboratory purified sAC protein from 950 rat testes [30,31]. Purified sAC was insensitive to G-protein or FSK stimulation and was activated by HCO_3^- (described in more detail below) [32].

sAC purification allowed for the molecular cloning of the mammalian sAC gene (*ADCY10*) and the identification of multiple protein isoforms generated by alternative splicing [30,33,34]. The *ADCY10* gene comprises 33 exons and predicts the 187 kDa full-length sAC (sAC_{fl}) protein, which contains two heterologous catalytic domains (C1 and C2) as well as multiple C-terminal regulatory domains, one of which has been identified as an autoinhibitory domain [35,36]. An alternatively spliced isoform which skips the 12th exon of the *ADCY10* gene introduces a premature stop codon to generate a 48 kDa 'truncated' splice variant (sAC_t) [34]. sAC_t corresponds to the sAC protein originally purified from rat testes and comprises the C1 and C2 catalytic domains of sAC [30]. Both isoforms are stimulated by HCO_3^-, but sAC_t has higher specific activity than sAC_{fl} [30]. This difference in activity is due to the presence of the autoinhibitory domain in sAC_{fl}, although the exact mechanism of autoinhibition remains unknown [35]. An additional molecularly characterized, alternatively spliced sAC isoform contains a ciliary targeting sequence and a single catalytic domain (C2) [33]. Sequence analysis of *ADCY10* revealed that C1 and C2 are closely related to the catalytic domains of ACs from Cyanobacteria, which evolved over 3 billion years ago [30].

Genetic ablation of the *ADCY10* gene ultimately confirmed that sAC is responsible for the testis-derived soluble AC activity originally observed by Braun [37,38]. Prior to its molecular isolation, soluble AC activity was believed to be abundantly expressed only in the testis [30]. It has since been shown that sAC is ubiquitously expressed and can be found in a wide variety of tissues [39]. sAC is known to be present in the cytoplasm as well as within cellular organelles [4], including the nucleus [19] and the mitochondrial matrix [4,20–22]. As discussed above, intracellular cAMP signalling microdomains depend upon an AC that can produce the second messenger away from the plasma membrane. In several cellular compartments, sAC was demonstrated to be that source [19–22].

3. Biochemical properties of sAC

Like all mammalian ACs, the catalytic mechanism of sAC requires the binding of two divalent cations in the active site [40,41]. sAC can use either Mn^{2+} or Mg^{2+} as metal cofactors though the activity of sAC is much greater in the presence of Mn^{2+} [42]. Although Braun originally discovered the soluble AC activity due to its preference for Mn^{2+} [23], it is unclear whether the intracellular concentration of Mn^{2+} would support sAC activity in mammalian cells. Studies performed with purified sAC_t revealed the biochemical properties that are responsible for this selectivity; in the presence of Mg^{2+}, sAC exhibits a much higher K_m for substrate ATP (K_m of approx. 1 mM with Mn^{2+} versus $K_m > 10$ mM with Mg^{2+}) [42]. Mg^{2+}-dependent sAC activity can be enhanced if another metal cofactor, Ca^{2+}, replaces the second Mg^{2+} in the active site. Ca^{2+} is better at coordinating ATP than Mg^{2+}; thus, this replacement results in a decrease in the K_m for substrate ATP (from 10 mM to approx. 1 mM) and an overall stimulation of sAC activity at cellular levels of ATP [41–43]. This effect is physiologically relevant, as activation of sAC in response to an increase in intracellular levels of Ca^{2+} was observed in several systems [44–48]. Even when sAC is fully activated, its affinity for ATP (K_m of approx. 1 mM) is much lower compared to that of tmACs ($K_m = 10$–100 µM) [13]. Due to its low affinity, sAC is not saturated with substrate at physiological ATP concentrations (approx. 1–3 mM). This property allows sAC activity to vary with physiologically relevant fluctuations in ATP concentrations, thus allowing it to function as an ATP sensor within a cell [49].

4. Bicarbonate stimulation: mechanism of action

HCO_3^- directly stimulates Mg^{2+}-dependent human sAC activity with an EC_{50} of 11–12 mM [41,42], which matches

the normal intracellular concentration of HCO_3^- [50–52]. HCO_3^- and Ca^{2+}, the other physiological sAC activator, are synergistic; together they greatly increase the level of Mg^{2+}-dependent sAC activity. Biochemical characterization of HCO_3^--induced sAC activation suggested that the mechanism of action involved the direct binding of HCO_3^- to sAC protein [32]. *In vitro*, HCO_3^- regulation was shown to be pH-independent and appeared to be due to HCO_3^-, but not CO_2, changes. HCO_3^- regulation is conserved in sAC-like cyclases from Cyanobacteria, and crystal structures of a cyanobacterial sAC homologue (CyaC) revealed that HCO_3^- induces closure of the active site and facilitates the recruitment of one of the metal cofactors [43]. The bicarbonate binding site (BBS) was ultimately identified when the crystal structure of human sAC was solved [41]. The identification of a BBS definitively showed that the mechanism of action for HCO_3^--induced sAC activation involved the direct binding of HCO_3^-.

Kinetic analysis revealed that HCO_3^- stimulates sAC via two mechanisms: it relieves substrate ATP inhibition and increases the V_{max} of the catalytic reaction [42]. The structural rationale for the second mechanism of activation, but not the first, was determined when the crystal structure of the mammalian sAC–HCO_3^- complex was solved [41]. All known mammalian ACs (sAC and tmACs) have two catalytic domains (C1 and C2) that pseudo-heterodimerize to form the catalytic centre at their interface. The active site contains several highly conserved residues essential for catalysis [41,43]. The C1–C2 interaction forms an additional pocket known as the pseudosymmetric site. The pseudosymmetric site does not contain the catalytic residues and, thus, is catalytically inactive [53]; instead, it is responsible for binding of small molecule activators (i.e. HCO_3^- in sAC and FSK in tmACs). The sAC pseudosymmetric site (i.e. the BBS) is significantly smaller than the tmAC pseudosymmetric site, making it unable to accommodate FSK and explaining the selectivity of FSK for tmAC. HCO_3^-, which is significantly smaller than FSK, is able to enter the BBS of sAC and bind between residues Lys95 and Arg176 [41]. The interaction between HCO_3^- and Arg176 within the BBS positions Arg176 towards the bound HCO_3^-, disrupting a salt bridge formed between Arg176 and a conserved catalytic residue, Asp99, in the apo-enzyme. The salt bridge between Arg176 and Asp99 in the apo-enzyme is an inhibitory interaction, which prevents Asp99 from coordinating one of the essential metal cofactors in the active site. The presence of HCO_3^- in the BBS therefore allows Asp99 to coordinate the active site metal and sAC to enter an active conformation. The HCO_3^--induced active site closure [43], which rearranges the bound substrate ATP into a conformation that facilities the formation and release of the reaction products, could explain the increased V_{max} of the reaction [42]. In addition, the presence of HCO_3^- in the BBS induces smaller active site rearrangements which are not yet fully characterized [41,53].

5. sAC functions

5.1. sAC as a bicarbonate sensor

5.1.1. Sperm

After being produced within the testis, sperm are stored in the cauda region of the epididymis. At this stage, the sperm are morphologically mature, but they are incapable of fertilizing an egg [54]. Beginning with ejaculation and continuing during transit through the female reproductive tract, sperm acquire the ability to fertilize an egg through a maturation process called capacitation [55]. Capacitation, which is essential for successful fertilization, is induced by HCO_3^- and dependent upon cAMP signalling [27]. As mentioned above, sAC was originally purified from 950 rat testes [30] and is known to be highly expressed in testis, specifically in male germ cells [56], and in sperm [37,57,58]. Both pharmacological inhibition and genetic ablation experiments demonstrate that sAC is the source of the second messenger responsible for the majority, if not all, of cAMP signalling that occurs within sperm [37,38,59–61].

While stored in the cauda region of the epididymis, mature sperm are in an environment with a HCO_3^- concentration that is significantly lower than standard extracellular levels (i.e. 2–7 mM instead of 25 mM) [62]. Once sperm leave the epididymis, the normal extracellular HCO_3^- in seminal fluid activates sAC and induces capacitation [63]. The essential role of sAC in HCO_3^--induced capacitation was demonstrated both genetically and pharmacologically. Male, but not female, sAC knockout (KO) mice are sterile, and sperm from these mice have defects in motility, fail to capacitate, and are thus incapable of fertilizing an oocyte *in vitro* [37,38,60]. Likewise, incubating sperm from wild-type (WT) mice with either of two, molecularly distinct, sAC-specific inhibitors, KH7 [37] or LRE1 [61], results in similar defects. sAC's function in sperm is also genetically confirmed in humans. A frameshift mutation in *ADCY10* was recently identified as the cause of infertility in two adult men [64]. Similar to sAC KO and sAC-inhibited mouse sperm, the sperm from these two individuals have defects in motility. Motility was restored by addition of cell-permeable cAMP analogues, confirming the defect was caused by insufficient levels of intracellular cAMP as a result of sAC loss.

5.1.2. Eye

Aqueous humour (AH) is a watery, nutrient-filled fluid found in both the anterior and posterior chambers of the eye. AH is secreted from the ciliary body (CB), and is continuously drained from the eye via several drainage routes, with the trabecular meshwork being responsible for a majority of the drainage. The balance between the rate of AH production (inflow) and the rate of AH drainage (outflow) determines intraocular pressure (IOP). Abnormal IOP can lead to the development of eye disorders; i.e. elevated IOP is a major risk factor for glaucoma [65] and reduced IOP can lead to phthisis bulbi (shrunken eye) [66]. Pharmacologic and genetic tools revealed that sAC regulates IOP. sAC KO mice as well as mice treated with either of the sAC-specific inhibitors, KH7 [67] or LRE1 [68], have elevated IOP. In sAC KO mice or sAC inhibitor-treated WT mice, the elevated pressure is due to decreased outflow of AH without significant changes to inflow [67]. The exact mechanism by which sAC controls outflow of AH remains unknown, but is thought to originate in the CB [69]. In contrast to the trabecular meshwork, which does not appear to contain sAC protein or sAC activity, the CBs of humans and pigs express high levels of sAC protein and contain measurable sAC activity [67,70]. In the CB, sAC is thought to act as a HCO_3^- sensor. In CB cells, application of a carbonic anhydrase inhibitor (CAI) increases intracellular HCO_3^- which stimulates sAC-dependent cAMP

production [71]. Interestingly, CAIs are a widely used treatment for glaucoma [65], suggesting that CAIs reduce IOP by increasing intracellular HCO_3^- levels and stimulating sAC activity in CB cells.

5.1.3. Astrocytes

sAC is also abundantly expressed in astrocytes, support cells of the brain [72]. Astrocytes produce and store glycogen [73] which they break down into lactate [74]. The lactate is supplied to neurons to be used as an energy source, making astrocytes an important contributor to energy efficiency in the brain [74–76]. Astrocytic sAC plays an important role in this metabolic coupling. Following neural activity, extracellular concentrations of potassium ($[K_e^+]$) are high. Both in cultured astrocytes and in brain slices, the elevated $[K_e^+]$ stimulates transport of HCO_3^- into astrocytes [72,77–80], which activates astrocytic sAC [72]. The resultant increase in cAMP promotes the breakdown of glycogen, increasing production of lactate [72]. These effects are blocked by sAC-specific inhibitors, but not by the tmAC-selective inhibitor dideoxyadenosine (ddAdo). Thus, HCO_3^- regulation of sAC is important for the stimulation of astrocytic glycogenolysis and lactate production following neural activity.

6. sAC also functions as a carbon dioxide and pH sensor

Although sAC activity is regulated directly by HCO_3^- *in vitro*, in cellular systems sAC also responds to changes in levels of carbon dioxide (CO_2) and protons (H^+). This responsivity is due to the ubiquitous presence of carbonic anhydrases (CAs), which catalyse the instantaneous equilibration of CO_2, HCO_3^- and protons (H^+). For this reason, sAC functions as a physiological intracellular pH (pH_i), CO_2 and HCO_3^- sensor, responding to changes in these factors via its activator HCO_3^-.

6.1. sAC as a carbon dioxide sensor

6.1.1. Mitochondria

Inside the mitochondrial matrix, the tricarboxylic acid (TCA) cycle produces electron donors for oxidative phosphorylation (OXPHOS). OXPHOS, which is responsible for generating a majority of cellular ATP, is dynamically regulated by post-translational modifications [81,82], including cAMP-dependent phosphorylation of mitochondrial enzymes [21,83]. Initially, the discovery of cAMP-dependent regulation was enigmatic; membrane-permeable cAMP added to cells increased oxygen consumption and ATP generation, but stimulating tmACs to produce cAMP in the cytoplasm had no effect [20]. The appreciation that mitochondrial sAC synthesizes cAMP inside the mitochondrial matrix resolved the conundrum of how membrane-impermeable cAMP was able to regulate its effector proteins inside the mitochondrial matrix [20,21,84]. As it produces electron donors, the TCA cycle also generates CO_2. Due to the presence of CAs in the mitochondrial matrix [85,86], this CO_2 is nearly instantaneously converted to HCO_3^-. Matrix-localized, HCO_3^--regulated sAC generates cAMP which increases electron transport chain and OXPHOS activities, resulting in increased ATP production [20]. Thus, CO_2-dependent sAC activity links TCA cycle flux with OXPHOS activity [83,87,88]. In addition to CO_2-dependent regulation, this intramitochondrial sAC signalling cascade is responsive to calcium released from intracellular stores [21,84]. The effects of intramitochondrial sAC-generated cAMP are reversible, and phosphodiesterase 2A (PDE2A) was identified as the intramitochondrial PDE responsible for regulating cAMP levels and OXPHOS activity [89].

6.1.2. Bronchi

Bronchi remove contaminants from inhaled air via a mechanism called mucociliary clearance (MCC) in which cilia lining the airway epithelium beat to propel unwanted material out of the lungs. sAC was identified in airway epithelium, where it is specifically localized to the axoneme, the microtubule-based cytoskeletal structure that forms the core of cilia and regulates ciliary beat frequency [90]. Baseline ciliary beat frequency (CBF) and changes in CBF are mediated by cAMP. CO_2/HCO_3^- exposure increases cAMP in cilia and stimulates CBF, and sAC-specific inhibitors, but not tmAC-selective inhibitors, block these effects [90]. sAC-dependent control of CBF was confirmed genetically. sAC KO mice lack the CBF regulation seen in WT mice, and recombinant expression of sAC targeted to cilia using a specific ciliary targeting sequence, but not cytoplasmic sAC, rescued sAC-dependent CBF regulation [33]. Thus, sAC in bronchi serves as a CO_2 sensor modulating ciliary beat.

6.2. sAC as an extracellular pH sensor

6.2.1. Epididymis

Acidification of the lumen of the cauda region of the epididymis is essential for sperm maturation and storage. Acidification is accomplished by the clear cells of the epididymis, which secrete protons from their apical pole lining the lumen [91,92]. Clear cells possess high levels of the vacuolar-type H^+-ATPase (V-ATPase) proton pump. Basally, the luminal pH is acidic, and the V-ATPase proton pump is actively recycled between intracellular vesicles and the apical plasma membrane. At alkaline luminal pH, apical membrane surface area increases, which allows more V-ATPase proton pump to accumulate on the apical membrane from sub-apical vesicles. In turn, this accumulation stimulates proton secretion to acidify the lumen. This process is dependent upon carbonic anhydrase activity, suggesting a role for intracellular HCO_3^- [93]. HCO_3^--regulated sAC localizes to the clear cells of the epididymis, and cell-permeable cAMP increases V-ATPase surface expression. Importantly, inhibition of sAC blocks the elevation of cAMP and accumulation of V-ATPase on the apical surface of clear cells. Thus, at alkaline luminal pH, carbonic anhydrase-dependent stimulation of sAC increases apical membrane V-ATPase accumulation.

6.2.3. Kidney

The body's acid–base status is regulated via proton or HCO_3^- secretion in the collecting duct of the kidney. Analogous to the mechanism that exists in the epididymis, sAC is thought to play a parallel pH-sensing role in the intercalated cells (ICs) of the kidney collecting duct. ICs can respond to changes in extracellular acid–base status by modulating proton secretion. While sAC was shown to be expressed in many segments of the kidney tubule [93], in renal ICs, it colocalizes with the V-ATPase [94]. As in the epididymis, in Type-A ICs, sAC is thought to be involved in the carbonic anhydrase-dependent increase in microvilli and translocation

of V-ATPase to the apical membrane, as well as the resultant increase in proton secretion during periods of acidosis.

6.3. sAC as an intracellular pH sensor

6.3.1. Lysosomes

The endo-lysosomal pathway is the crucial intracellular pathway that follows the processes of endocytosis and autophagy [95,96]. Along the endosomal pathway, early endosomes mature, become late endosomes, and eventually merge with lysosomes. The acidification process is essential for efficient lysosomal protease function and breakdown of endo-lysosomal contents [97]. sAC is necessary for proper lysosomal acidification in a variety of cell types. Pharmacological and genetic ablation of sAC increases lysosomal pH [98]. Furthermore, multiple assays showed that in sAC KO or sAC-inhibited WT cells, lysosomal degradative capacity is reduced and autophagic vacuoles accumulate [98]. One possible mechanism underlying this phenotype is that sAC activity is regulated by local increases in HCO_3^- due to changes in intracellular pH near acidifying lysosomes.

6.3.2. Melanosomes

Melanin, which determines skin colour, is synthesized in melanocytes inside a specialized lysosome-related acidic organelle called a melanosome. The rate-limiting step in melanin synthesis involves tyrosinase, a pH-sensitive enzyme. At basic pH, tyrosinase is active, which results in increased melanin synthesis [99]. Reduction of sAC activity, via either genetic or pharmacological inhibition, increases melanosome pH, which stimulates tyrosinase activity and accumulation of melanin [99].

7. Additional sAC functions

sAC has additional functions which have been demonstrated both genetically and pharmacologically, but which have not, at least by currently available findings, been directly ascribed to its CO_2/HCO_3^-/pH-sensing capabilities. In addition to CO_2/HCO_3^-/pH, sAC activity can be regulated by Ca^{2+}, ATP and multiple regulatory domains located in the C-terminus of the protein [35,36,44–49]. A recent role for sAC has been uncovered in the liver. Non-alcoholic steatohepatitis (NASH) is an advanced form of hepatic steatosis that is mainly characterized by the presence of fibrotic scarring on the liver. sAC was shown pharmacologically and genetically to be essential for development of fibrotic scarring in response to high cholesterol in animal models of NASH [100]. sAC has also been ascribed immune functions in different contexts. sAC is required for transendothelial migration of leucocytes, the process by which leucocytes migrate through the endothelium to gain access to sites of infection [101]. sAC has also been identified to play a role in cAMP-dependent regulation of inflammasome functions [102–104]. Whether these sAC functions depend upon sAC's CO_2/HCO_3^-/pH-sensing capabilities or an alternative sAC regulatory mechanism awaits further studies.

8. Conclusion

This review details a number of sAC functions that have been demonstrated both pharmacologically and genetically. Despite this wide range of sAC functions, two different sAC KO mouse strains [37,105] and humans homozygous for a rare frameshift mutation that results in a premature termination of the *ADCY10* gene [64] exhibit male-specific sterility with no other readily observable phenotypes [37,38,105]. Other identified phenotypes in sAC KO mice were more subtle and required deeper investigation to uncover. In contrast to sperm, where sAC-generated cAMP is required to initiate capacitation in an 'all-or-nothing' manner, the 'somatic' functions of sAC-generated cAMP appear to modify the amplitude or timing of pathways. For example, in the mitochondrial matrix, sAC-generated cAMP does not turn on or off the electron transport chain; instead it acts as a rheostat to control how much ATP is generated [20,22,83,106]. Similarly, sAC-generated cAMP regulates beating frequency (not whether there is a beat) in airway cilia [90] and the rate and efficiency of lysosomal acidification [98].

Discovery of HCO_3^--regulated sAC revealed a mechanism by which cells and organisms can respond to changes in HCO_3^-, CO_2 or pH and defined CO_2/HCO_3^-/pH as physiological signals. Future studies of sAC, including characterization of sAC functions not yet known to depend upon CO_2/HCO_3^-/pH sensing and identification of novel sAC functions, will further illuminate the biology of CO_2/HCO_3^-/pH signalling.

Data accessibility. This article has no additional data.

Authors' contributions. T.R., S.J., J.B. and L.R.L. all wrote and edited the manuscript together.

Competing interests. J.B. and L.R.L. own equity interest in CEP Biotech which has licensed commercialization of a panel of monoclonal antibodies directed against sAC.

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