Biochimica et Biophysica Acta 1842 (2014) 2601–2609

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

Review

Aldosterone signaling and soluble adenylyl cyclase—A nexus for the kidney and vascular endothelium☆

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ARTICLE INFO

Article history:
Received 3 April 2014
Received in revised form 23 May 2014
Accepted 28 May 2014
Available online 5 June 2014

Keywords:
Endothelial stiffness
Bicarbonate
cAMP
Gene expression
Spironolactone

ABSTRACT

The steroid hormone aldosterone regulates the reabsorption of water and ions in the kidney and plays a central role in blood pressure regulation and homeostasis. In recent years, the vascular endothelium has been established as an important aldosterone target organ with major implications in renal and cardiovascular health and disease. Different lines of evidence suggest that the calcium- and bicarbonate-activated soluble adenylyl cyclase (sAC) is a novel mediator of aldosterone signaling in both the kidney and vascular endothelium. This review summarizes our current understanding of the molecular mechanisms of sAC gene expression regulation in the kidney and vascular endothelium and outlines the potential clinical implications of sAC in chronic kidney disease and cardiovascular disease. This review is part of a special issue entitled: The role of soluble adenylyl cyclase in health and disease. This article is part of a Special Issue entitled: The role of soluble adenylyl cyclase in health and disease.

1. Introduction

In 1999, the soluble adenylyl cyclase (sAC) (OMIM #605205) was identified as a new, distinct and soluble class of mammalian adenylyl cyclase, insensitive to G protein or forskolin regulation [1]. The human sAC gene *ADCY10* contains 33 exons covering approximately 104 kb on chromosome 1q23.3-q24 [2,3] and encodes different sAC isoforms, localized in the nucleus, cytoplasm and mitochondria, with distinct tissue functions [4–16]. Two heterologous sAC catalytic domains (C1 and C2) are known and up to 20-fold higher cAMP-forming activity has been reported for the truncated ~50 kDa sAC isoform compared to the full-length form (see review by C. Steegborn in this issue for a schematic representation) [1]. The sAC full-length form contains an additional non-catalytic, C-terminal, autoinhibitory region which functions to lower the enzyme's Vmax without altering its substrate affinity [17]. From an evolutionary point of view, the catalytic sAC domains show higher conformity with cyanobacterial adenylyl cyclase than with mammalian transmembrane adenylyl cyclases (tmACs) [18], underlining its unique rank among the otherwise homologous enzymes. The C-terminal end of the sAC full-length form comprises several putative regulatory domains, including an autoinhibitory region, a canonical P-loop, and a leucine zipper sequence [19]. sAC is an evolutionarily conserved bicarbonate sensor [18,20] and three other important intracellular messengers, namely CO_2, ATP, and Ca^{2+} have been reported to regulate sAC [18,21,22]. Due to the broad range of cellular processes that potentially involve sAC but also other adenylyl cyclases, the exact role of sAC is only partly understood. This review will focus on sAC in renal and vascular health and disease, the role of sAC in aldosterone signaling and nuclear sAC as a transcriptional regulator [8,23].

2. Aldosterone

The circulating steroid hormone aldosterone is predominantly produced in the cortex of the adrenal gland [24] and was first isolated and characterized in 1953 [25]. Aldosterone plays a central role in the regulation of blood pressure mainly by acting on the epithelial sodium channel (ENaC) in epithelial cells of the renal distal tubule, increasing sodium reabsorption essential for the maintenance of body salt and water homeostasis [26]. The key regulators of aldosterone production are the adrenocorticotropic hormone (ACTH), potassium and angiotensin II (Ang II) with calcium as a critical mediator [27]. Ang II is a product of the complex systemic and evolutionarily well conserved renin–angiotensin–aldosterone system (RAAS), which is the principal volume-regulatory effector in mammals and a major regulator of blood pressure in humans (Fig. 1) [28]. The key effector precursor molecule of the RAAS is angiotensinogen, a protein produced in the liver [29]. The unique kidney-derived aspartyl protease renin, produced by the juxtaglomerular apparatus, constitutes the rate-limiting and regulating step and cleaves angiotensinogen to a small peptide of 10 amino acids termed angiotensin

☆ This article is part of a Special Issue entitled: The role of soluble adenylyl cyclase in health and disease.

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http://dx.doi.org/10.1016/j.bbadis.2014.05.036
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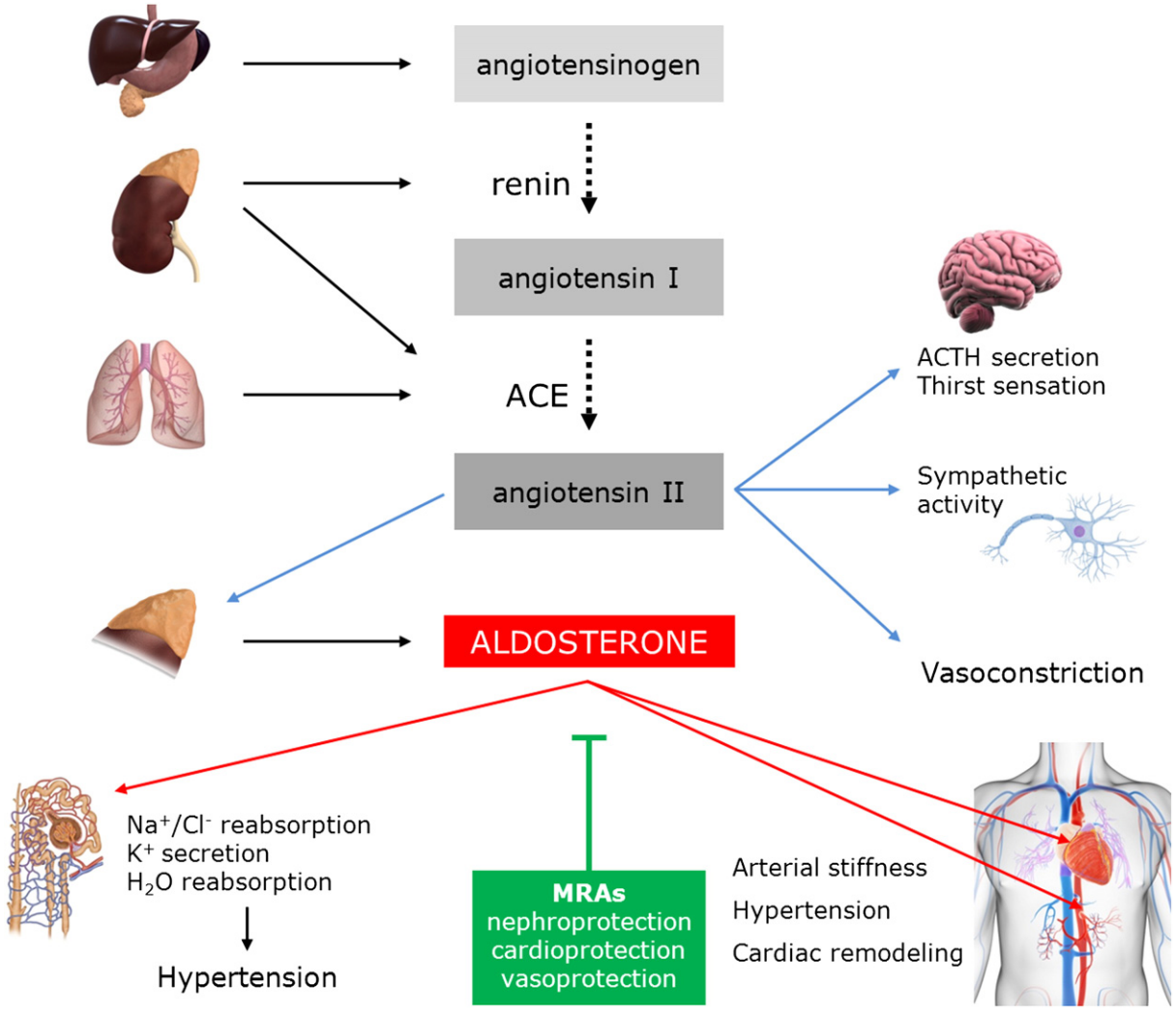

Fig. 1. Regulation of aldosterone secretion and systemic aldosterone effects. Schematic representation of the major components of the renin–angiotensin–aldosterone system (RAAS). Angiotensinogen of the liver is hydrolyzed by kidney-derived renin in the blood. The resulting angiotensin I is processed by the angiotensin converting enzyme (ACE), which is produced by lung and kidney to the highly active effector peptide angiotensin II. Angiotensin II regulates the expression of the adrenocorticotropic hormone (ACTH) in the pituitary gland, thirst sensation, sympathetic activity and mediates vasoconstriction. In the adrenal gland, angiotensin II stimulates the release of aldosterone, which regulates Na^+/Cl^- reabsorption and K^+ secretion in the nephron. Elevated levels of aldosterone may lead to arterial stiffness, cardiac remodeling and hypertension. Deleterious aldosterone actions can be antagonized by clinically used mineralocorticoid receptor antagonists (MRAs) with nephroprotective, vasoprotective and cardioprotective effects.

I (Ang I) within the blood [30]. The downstream and endothelial (mainly pulmonary endothelium)-derived matrix metalloproteinase angiotensin converting enzyme (ACE) subsequently cleaves Ang I to the highly active effector peptide Ang II, which in turn releases aldosterone from the adrenal cortex [28]. In the brain, the RAAS-induced Ang II stimulates thirst and salt appetite [31]. Besides the strong impact on blood pressure and hypertension [32], RAAS dysregulation with increased aldosterone secretion has been associated with chronic kidney disease (CKD) and cardiovascular disease (CVD), including endothelial dysfunction and arterial stiffness [33–39]. The important role of individual components of the RAAS in the development of hypertension has also been underlined by findings on the genetic and genomic level. Genes of the RAAS such as the aldosterone synthase gene (*CYP11B2*), the mineralocorticoid receptor (MR) gene (*NR3C2*), the angiotensinogen gene (*AGT*) and genes that mediate or regulate renal sodium handling such as ENaC (*SCNN1B/G*) subunits have been identified to cause monogenic forms of hypertension [30,40,41] and have been reported from genome-wide association studies to be associated with blood pressure phenotypes [reviewed by 42]. Despite local aldosterone expression in the adrenal glands, extra-adrenal production of aldosterone by the aldosterone synthase CYP11B2 has been identified in the heart, blood vessels, and brain [43].

3. The mineralocorticoid receptor (MR)

Aldosterone exerts its effects via the MR (nuclear receptor subfamily 3, group C, member 2; NR3C2), which belongs to the nuclear receptor superfamily and functions as a ligand-dependent transcription factor [44]. The MR mediates aldosterone effects on a variety of target tissues, including the distal tubule of the kidney, the cardiovascular and central nervous systems, the distal colon, the skin and sweat glands, as well as brown adipose tissue [45,46]. MR selectivity is regulated on multiple levels and several mechanisms are involved to generate distinct MR isoforms and protein variants [reviewed by 47]. In humans, alternative transcription of 5′-untranslated exons 1alpha and 1beta is known to generate two different mRNA isoforms, hMRalpha and hMRbeta and at least two different protein variants, MRA and MRB have been reported, which display distinct transactivation capacities *in vitro*. In addition, alternative MR-splicing occurs and post-translational modifications of the MR including phosphorylation, acetylation, ubiquitination and sumoylation have been identified [47]. While the exact function of MR isoforms and post-translational modifications is still under debate, these mechanisms are likely to affect MR transcriptional regulation and MR interaction with target molecules and coactivators as well as MR turnover and subcellular trafficking. Berger et al. underlined the

importance of the MR for renal sodium and water handling by generation of MR-deficient mice [48]. MR-deficient mice showed hyperkalemia, hyponatremia and a strong increase in renin, Ang II, and aldosterone plasma levels with symptoms of pseudohypoaldosteronism. Increasing evidence from *in vitro*, *in vivo* and clinical studies suggests that in addition to its role in the regulation of sodium handling, functional MR is expressed in a broad range of non-epithelial tissues, in particular the cardiovascular system, contributing to hypertension, vascular inflammation, remodeling and atherogenesis [49]. Strong evidence for the deleterious actions of MR in the vasculature can be derived from smooth muscle cell-specific MR-deficient mice, which lack many aspects of cardiovascular aging, such as increased blood pressure and blood pressure responsiveness to Ang II, cardiac hypertrophy and increased Ang II-dependent vasoconstriction [50]. Using this smooth muscle cell-specific MR-deficient model, it has recently been demonstrated that vascular smooth muscle MR is essential for injury-induced vascular fibrosis, for aldosterone-induced vascular remodeling even under physiological aldosterone levels and for enhanced vascular remodeling in response to aldosterone excess [51]. In addition, Galmiche et al. showed that vascular smooth muscle MR is required for increased arterial stiffness in response to aldosterone and high salt-induced hypertension [52]. MR-mediated signaling also affects vascular endothelial cells, in that it modulates endothelial stiffness and thus endothelial function including the expression of the vasodilator nitric oxide (NO), regulating the vascular tone [53–56]. In addition to the 'classical' genomic aldosterone effects, rapid and non-genomic MR-dependent aldosterone actions, independent of transcription and translation, have been reported in the renocardiovascular system [57–59]. Deleterious non-genomic MR actions may lead to endothelial dysfunction, inflammation, as well as cardiac remodeling [reviewed by 59]. Notably, rapid effects of aldosterone have been suggested to be linked to activation of adenylyl cyclases and cAMP production [58,60,61]. Since aldosterone and the glucocorticoid cortisol have a comparable affinity for the MR, an additional mechanism exists to confer aldosterone-specific actions. The 'gatekeeper' enzyme 11beta-hydroxysteroid dehydrogenase (11beta-HSD) leads to the conversion of active cortisol to receptor-inactive cortisone so that aldosterone gains access to the MR [62]. Despite broad acceptance of this concept, results from *in vivo* binding studies suggest that the mechanism involving 11beta-HSD may be far more complex [63]. It seems that 11beta-HSD is not efficient in reducing the cortisol level to the required threshold and glucocorticoids can occupy but not activate the MR in the presence of 11beta-HSD. Thus, an additional (rather than alternative) mechanism has been suggested [64], involving 11beta-HSD-derived NADH, which is a potential transcriptional inhibitor [65] and might keep glucocorticoid-occupied MR in an inactive state.

4. Mineralocorticoid receptor antagonists (MRAs)

4.1. Clinical relevance

Large prospective cross-sectional studies have reported that circulating aldosterone levels are independently associated with cardiovascular morbidity and mortality [66–68]. These studies also suggest that even aldosterone concentrations within the 'normal' range could result in clinically relevant MR activation, which may be prevented via MR signaling blockade by MRAs to prevent long-term pathophysiological changes. The first MRA, spironolactone, was developed over 50 years ago with principal use in primary and secondary hyperaldosteronism. Spironolactone was followed by eplerenone, which is less potent but MR-selective [69] with shorter half-life and inactive metabolites. Today, MRAs are guideline-recommended drugs for patients with moderate to severe heart failure (HF) [70]. Large clinical trials including the Randomized Aldactone Evaluation Study (RALES) [71] and the Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS) [72], demonstrated that MRAs can significantly reduce morbidity and mortality in HF patients. These trials were followed by the Eplerenone in Mild Patients Hospitalization and Survival Study in Heart Failure (EMPHASIS-HF), which included patients with mild HF and documented the efficacy and safety of MRAs in these patients [73]. Notably, the protective effect of spironolactone was clearly demonstrated in the RALES study, although only slightly elevated circulating aldosterone levels were observed [49]. This may be explained by the fact that in conditions of tissue damage, in contrast to normal physiological conditions, glucocorticoids become functional MR agonists [64].

Aldosterone-induced and blood pressure-independent renal injuries such as renal inflammation, renal fibrosis, proteinuria as well as glomerular and podocyte injury have also been identified as potential therapeutic uses of MRAs [74]. Consistently, MRAs have been suggested as a therapeutic strategy for patients with CKD [75]. Current clinical guidelines have, however, included cautionary notes for the prescription of MRAs in CKD patients. While the RALES investigators included HF patients with serum creatinine concentrations up to 2.5 mg/dl, the incidence of hyperkalemia was low [71]. A subsequent review of 'real-world' practice demonstrated a significant increase in hyperkalemia when the combination of RAAS blockade and MRAs had been prescribed [76]. This creates a therapeutic dilemma in patients with HF and CKD, as MRAs might be beneficial in preventing mortality due to HF but may have considerable side effects especially in patients with low estimated glomerular filtration rate (eGFR). Therefore, low-dose MRAs in patients with reduced kidney function (GFR < 45 ml/min/1.73 m^2; GFR < 30 ml/min/1.73 m^2 not indicated) and close monitoring of potassium levels are recommended [77]. MRAs (i.e. spironolactone) are also recommended in patients with therapy-resistant hypertension as an additional therapeutic option [69].

4.2. Mechanism of action

Spironolactone is effective at considerably low doses of 10 nM [78,79], which has led to the assumption that it is acting as an inverse MR agonist rather than a true MRA [64]. It might be due to this attribute that the cardioprotective effects and exact molecular mechanisms of spironolactone besides its function of 'blocking' MR signaling are still incompletely understood [80]. It seems conceivable that MRAs have a broad range of pharmacological effects since MR-dependent signaling is diverse and a multitude of downstream target genes exists. Notably, data from our lab suggests that spironolactone inhibits the translocation of sAC into the nucleus in renal and endothelial cells (own unpublished data). This observation provides an essentially new basis to discuss potential MRA actions.

5. sAC in the kidney

5.1. sAC and bicarbonate

While the general role of adenylyl cyclases in salt and water homeostasis [reviewed by 81] and in renal cAMP formation [reviewed by 19] is well established, detailed information on the individual and specific functions of the diverse adenylyl cyclase enzymes is sparse. A broad range of tmACs and the sAC are expressed in the kidney, which yields a high number of potential cellular cAMP sources [82]. In a considerable number of experiments, the effects of cAMP on hormonal (i.e. mineralocorticoid or glucocorticoid) receptor signaling have been analyzed using synthetic cAMP derivatives or forskolin [reviewed by 83]. However, these experiments were not designed to reveal the *in vivo* cAMP origin (i.e. the specific adenylyl cyclase isoform).

sAC is different compared to other adenylyl cyclases with respect to certain activators, which allows for the allocation of cellular functions and pathophysiological processes to this enzyme. An early but indirect evidence for sAC activity in the kidney was reported in a study that described bicarbonate-stimulated cAMP forming activity in rat kidney homogenates [84]. The origin of the cAMP remained elusive until sAC

was isolated and characterized as bicarbonate sensor [1,19]. Data presented by Pastor-Soler et al. indicated that sAC is involved in the regulation of renal proton secretion and a potential sensor for monitoring acid–base homeostasis by responding to bicarbonate concentration [85,86]. The group provided evidence that sAC-mediated increases in intracellular cAMP levels resulted in plasma membrane accumulation of vacuolar proton pumping ATPase (V-ATPase), a protein involved in renal proton secretion [84]. By immunofluorescence of normal rat kidney cortex, Paunescu et al. detected colocalization of sAC and the V-ATPase in the apical and basolateral plasma membrane of alpha- and beta-intercalated cells (ICs), which regulate bicarbonate reabsorption and proton (alpha-ICs) or bicarbonate secretion (beta-ICs) [87]. These experiments also suggest that sAC, despite its general soluble character and thus predominant cytoplasmic localization, is concentrated in functional complexes under certain physiological conditions. Interestingly, the sAC-dependent V-ATPase mobilization has been discussed as part of an evolutionary conserved mechanism for pH sensing involving sAC [88], since a similar mechanism of pH homeostasis has been described in shark [89]. In ICs, sAC seems to be localized in domains associated with the apical or basolateral plasma membrane in close proximity to its potential target molecule V-ATPase and coimmunoprecipitation suggested that the V-ATPase interacts specifically with the 50 kDa sAC isoform. It seems conceivable that subcellular sAC concentrations in processes related to bicarbonate homeostasis might be regulated by scaffold proteins which assemble multiple signaling enzymes, receptors or ion channels into functional complexes [90]. This is also supported by studies which used a cellular cAMP monitoring system based on the expression of sAC to show that phosphodiesterases and A-kinase anchoring proteins (AKAPs) are involved in nuclear protein kinase A (PKA) responses [91], as well as the observation that sAC compartmentalizes in signaling microdomains [92].

In 2013, researchers of the Chronic Renal Insufficiency Cohort (CRIC) study reported an association between serum bicarbonate misbalance and adverse outcomes in patients with CKD, in that a nonlinear relationship between bicarbonate levels and HF outcomes existed [93]. In a subset of participants without CVD at baseline, a 22% higher risk of HF was detected for every 1-mEq/L increase in serum bicarbonate level >24 mEq/L. In addition, serum bicarbonate level was independently associated with renal outcome defined as progression to end-stage renal disease or 50% eGFR reduction, even after adjustment of baseline values for eGFR and proteinuria [93]. The molecular mechanisms underlying these observations are not fully understood, but recent studies suggested that CKD patients without metabolic acidosis nevertheless have H^+ retention that increases plasma aldosterone levels, possibly mediating GFR decline [94]. Notably, aldosterone has been reported to increase V-ATPase-dependent proton secretion in intercalated cells of the outer medullary collecting duct, which may contribute to acid secretion and control of systemic acid–base homeostasis [95]. It seems conceivable that both aldosterone and bicarbonate may affect renal processes via sAC-dependent mechanisms.

5.2. sAC and calcium

In addition to the sensitivity of sAC to bicarbonate, calcium has been identified as a modulator of sAC activity, connecting sAC to bone mineral metabolism and the complex mechanism of systemic calcium regulation. Calcium, in contrast to bicarbonate, increases the sAC affinity for ATP-Mg^{2+} [21]. Therefore, calcium and bicarbonate synergistically activate sAC and small changes of either calcium or bicarbonate could lead to significant changes in cellular cAMP levels [21]. Several sAC splice variants have been detected in osteoclasts and osteoblasts [96] and experiments using the sAC inhibitor 2CE or anti-sAC-siRNA suggested that sAC is involved in inhibition of osteoclast differentiation at high bicarbonate levels [13]. This observation indicates that bicarbonate-sensing sAC is a physiologically relevant regulator of bone formation and/or reabsorption even if the exact role of sAC in osteoclastogenesis is still under debate since similar inhibitory effects during bicarbonate-free conditions have also been observed [19]. The involvement of sAC in systemic calcium regulation might be of clinical relevance since progression of impaired renal function is accompanied by increasing abnormalities of serum calcium, phosphate, parathyroid hormone (PTH) and vitamin D metabolites [77]. Robust data are available for elevated PTH levels, which have been associated with all-cause and cardiovascular mortality in patients with coronary artery disease [97] and end-stage renal disease [98]. Dysregulation of calcium and phosphate has been suggested to occur relatively later in the course of CKD compared to detectable changes of vitamin D and PTH and current guidelines recommend measuring serum levels of calcium, phosphate and PTH at least once in patients with GFR < 45 ml/min/1.73 m^2 to determine baseline values [77].

PTH is secreted by the parathyroid glands and acts to increase calcium levels in the blood by a complex mechanism in several target organs. In the kidney, PTH enhances reabsorption of calcium and magnesium, while it triggers a decrease in phosphate reabsorption thus leads to increased phosphate excretion in the urine [99]. In addition, PTH regulates the renal production of active 1,25-dihydroxy vitamin D, which in turn enhances the intestinal saturable active transport of calcium [100]. PTH also stimulates bone reabsorption resulting in calcium and phosphate release. In addition to low serum calcium levels as stimulus for PTH secretion, the clinically relevant impact of the RAAS on PTH secretion has been discussed [101] based on interventional studies including Ang II and aldosterone [102]. The study by Brown et al. also suggested that PTH may be regulated by aldosterone since reduced PTH and raised serum calcium levels were observed under MRA therapy [102]. The clinical relevance of this observation has been underlined by introduction of the EPATH (effect of eplerenone on parathyroid hormone levels in patients with primary hyperparathyroidism) study, which was designed to evaluate the influence of long-term MRA treatment in a population with primary hyperparathyroidism [99].

Several studies proposed that aldosterone may cause calcium wasting, particularly in the setting of dietary salt excess, although conflicting results have been reported and the underlying mechanisms remain largely unknown [103–107]. One potential explanation for the inconsistent reports on aldosterone-induced calcium wasting might involve individual genetic predispositions. Interestingly, a few reports on association of the *ADCY10* gene locus with calcium excretion exist, providing a potential genetic link between calcium, kidney and the vasculature. Initially, linkage analyses in three families with absorptive hypercalciuria have indicated an association with the *ADCY10* locus on chromosome 1q23.3-q24 [2]. Deep sequencing of genes located at this chromosomal site identified an association between hypercalciuria and six single nucleotide polymorphisms (SNPs) in the *ADCY10* gene, in that two SNPs were only detected in hypercalciuric cases. Bone mineral density (BMD) was lower as the number of associated alleles increased and osteoporosis was more frequent in individuals with at least one *ADCY10* SNP [3], confirming the relevance of this gene for calcium homeostasis. Based on a detected linkage of spinal BMD to chromosome 1q [108,109] and the reports mentioned above, Ichikawa et al. tested *ADCY10* as a candidate gene for association with BMD in healthy White Americans and detected a modest but significant effect of the *ADCY10* genotypes on BMD [110]. The results have recently been replicated by Cao et al. using an integrative data analysis approach to identify genes associated with osteoporosis [111]. These individual reports suggest that variations at the *ADCY10* locus may cause mild calcium wasting in urine, which could lead to the subsequently lowered BMD over time. The identification of the sAC coding gene *ADCY10* in these studies promote sAC from a simple bystander to a disease-causing candidate, which affects calcium homeostasis. Further studies are needed to evaluate whether the observed associations might also play a role in the calcium–phosphate–PTH–vitamin D axis in vascular calcification and CKD or aldosterone-dependent calcium wasting processes. These studies may also provide evidence if modulation of both sAC activity and aldosterone-induced

MR activation could prevent the calcium wasting properties of aldosterone.

5.3. sAC, ENaC and the Na^+/K^+-ATPase

The unique nature of sAC as bicarbonate sensor and the discovery of the specific pharmacological sAC inhibitor KH7 [112] allow for the distinction of molecular processes which involve sAC [113]. These specific sAC characteristics have been used and extended most recently by Sample et al. who attached a monomeric red fluorescent protein to the catalytically active form of sAC (sAC_t) [18] in combination with different localization signals to target subcellular sAC_t and thus monitor and manipulate cellular cAMP dynamics [91]. In 2008, Hallows et al. postulated that sAC may be an important regulator of sodium reabsorption in the kidney [5]. Using the specific sAC inhibitor KH7 and anti-sAC-siRNA, the group provided evidence that sAC inhibition leads to impaired basal trans-epithelial sodium currents in mouse cortical collecting duct cells (Fig. 2). This indicated a role for sAC in aldosterone-stimulated trans-epithelial sodium currents that depend on both ENaC and the Na^+/K^+-ATPase. Sodium reabsorption from urine in the distal nephron is controlled by aldosterone via surface expression and activity of the apical heterotrimeric ENaC [26]. With decreasing blood pressure or reduced circulating volume, aldosterone secretion increases, leading to subsequent ENaC activation in the collecting duct. The electrochemical driving force for ENaC is provided by the Na^+/K^+-ATPase [114]. The Na^+/K^+-ATPase is an integral membrane protein composed of an alpha and beta subunit, essential for maintaining the electrochemical gradients of Na^+ and K^+ across the plasma membrane [115]. Under physiological conditions, the Na^+/K^+-ATPase exchanges three intercellular Na^+ for two extracellular K^+ at the expense of one ATP. Besides the important mechanism of feedback control in sodium handling by intracellular Na^+, aldosterone actions independent of Na^+ on ENaC and Na^+/K^+-ATPase exist [114,115], which involve regulation on the transcriptional level. Based on the observations of Hallows et al. [5], we have recently identified a regulatory role of sAC on ENaC and Na^+/K^+-ATPase gene expression in that basal ENaC and Na^+/K^+-ATPase protein levels were significantly reduced by 24 h application of KH7 in renal epithelial cells *in vitro* [8]. This observation of

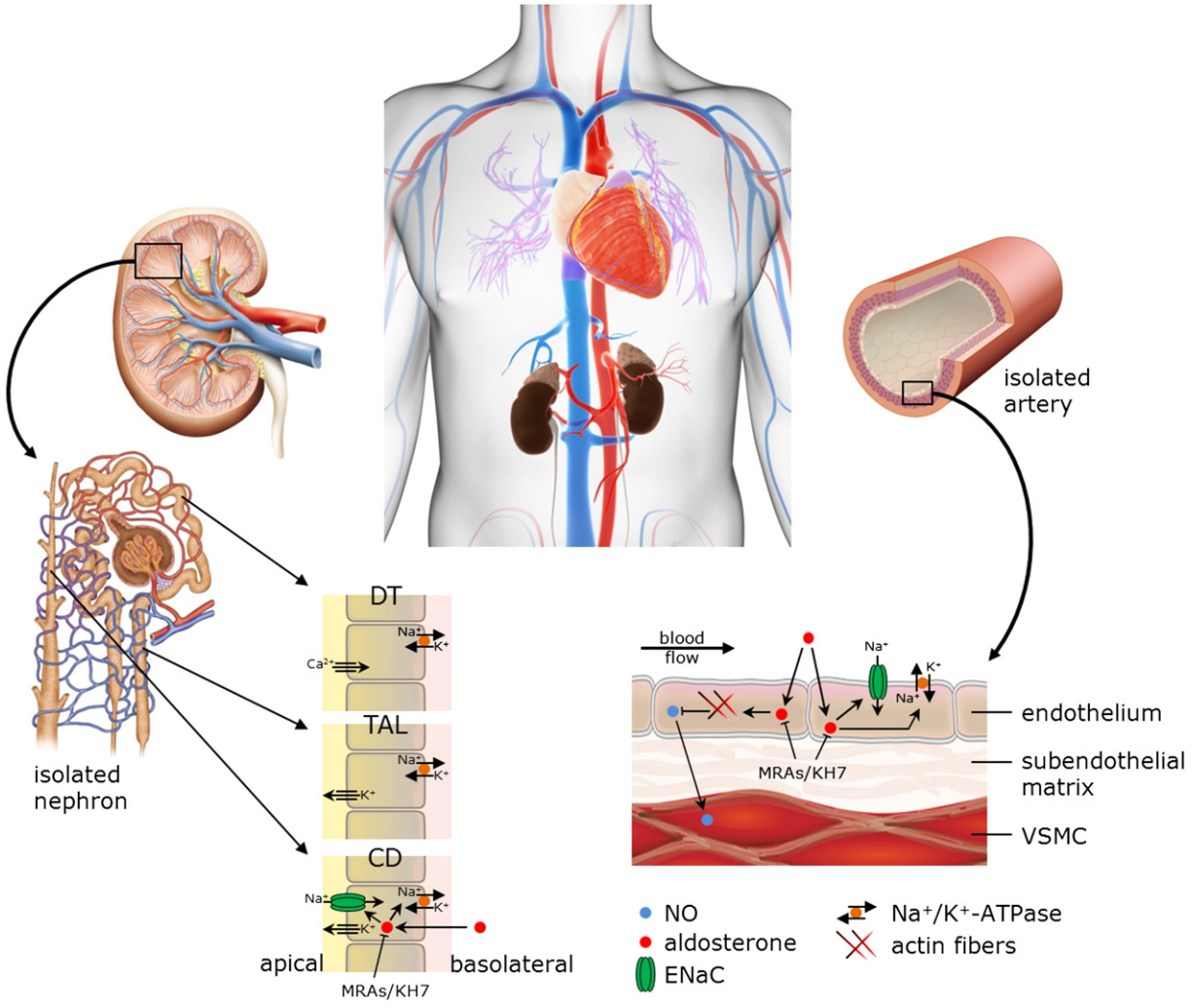

Fig. 2. Effects of aldosterone and sAC on renal epithelial and vascular endothelial cells. Renal sodium handling is controlled by aldosterone via surface expression and activity of the apical heterotrimeric epithelial Na^+ channel (ENaC), which promotes reabsorption of sodium from urine in the distal nephron. Aldosterone secretion increases with decreasing blood pressure or reduced circulating volume, leading to subsequent ENaC activation in the collecting duct (CD). Non-genomic aldosterone signaling stimulates rapid ENaC translocation from a vesicular pool to the apical membrane. In renal epithelial cells, basolateral Na^+/K^+-ATPase provides the electrochemical driving force for apical ENaC. Besides the CD, Na^+/K^+- ATPase is also highly active in the distal tubule (DT) and thick ascending limb (TAL). In the vascular endothelium, the expression of ENaC and Na^+/K^+-ATPase have been associated with endothelial stiffness in the presence of elevated aldosterone and Na^+ concentrations. Aldosterone also affects the actin fiber composition of endothelial cells, which leads to altered mechanosensitivity and decreased production of nitric oxide (NO) with impaired vascular smooth muscle (VSMC) cell relaxation and reduced vasodilation. In both, renal epithelial and vascular endothelial cells Na^+/K^+-ATPase and ENaC expression is regulated by aldosterone and sAC at the transcriptional level. In CD cells, aldosterone-stimulated transepithelial Na^+ current depends on sAC activity. In endothelial cells, sAC is involved in the regulation of actin fiber composition and endothelial stiffness. Deleterious aldosterone effects in the renal CD and vascular endothelium can be prevented by the mineralocorticoid receptor antagonist (MRA) spironolactone and the sAC inhibitor KH7.

sAC-dependent regulation of ENaC and the Na^+/K^+-ATPase gene expression also provided insight into a shared mechanism between the kidney and the vascular endothelium (Fig. 2).

6. sAC in the vascular endothelium

In recent years, endothelial ENaC and the Na^+/K^+-ATPase have been identified as important regulators of endothelial function [54,55,116]. Both, ENaC and the Na^+/K^+-ATPase, are involved in the salt-sensitive regulation of mechanical properties of the endothelial cell and thus affect endothelial stiffness which is crucial for the balance between endothelial function and dysfunction (Fig. 2). Aldosterone is a potent modulator of endothelial stiffness by altering the cellular actin composition and the cell's sensitivity to extracellular sodium changes [117,36]. The altered nanomechanic properties of endothelial cells in vascular endothelium determine nitric oxide (NO) release in that soft endothelial cells release more vasodilating NO than stiff endothelial cells [36], whereas the latter can be seen as the hallmark for endothelial dysfunction [118]. The endothelium-derived NO directly acts on vascular smooth muscle cells (Fig. 2), leading to vasodilation and decreased vascular tone [36,54,55]. Thus, the vascular endothelium is supposed to play a crucial role in maintaining overall arterial stiffness [119]. Patients' arterial stiffness can be assessed by pulse wave velocity (PWV) measurement and increased PWV is a strong and independent predictor of cardiovascular morbidity and all-cause mortality [120,121]. Besides its effects on endothelial actin fiber composition and NO production, aldosterone also regulates endothelial stiffness by the insertion of ENaC into the plasma membrane of the vascular endothelium [55,122,123], a mechanism first detected in renal epithelial cells. Consistently, MRAs have been shown to prevent overall arterial stiffness [119] and the application of spironolactone has been reported to prevent the 'stiff endothelial cell syndrome' [124]. Endothelial stiffness can be assessed *in vitro* and *ex vivo* by using the atomic force microscope (AFM) as a nanosensor [56,118]. Our group has recently reported that the expression of genes regulating endothelial stiffness depends on sAC [8]. Our observations suggest that sAC is involved in the regulation of the Na^+/K^+-ATPase alpha and beta subunit as well as ENaC alpha in endothelial cells. AFM measurements detected significant endothelial softening after treatment of endothelial cells *in vitro* with KH7. Furthermore, the significant aldosterone-induced stiffening of mouse aorta endothelial cells was prevented by sAC inhibition *ex vivo* and KH7-treated mouse aorta endothelial cells were significantly softer than untreated cells. These results indicated that sAC inhibition via the observed effects on gene expression, even in the presence of aldosterone, translate into an overall endothelial softening.

6.1. Transcriptional regulation by sAC

Interestingly, chromatin immunoprecipitation after sAC inhibition revealed altered interactions between transcription factors, including cAMP response element-binding protein (CREB) and promoter regions with active endothelial cAMP responsive elements (CRE) [8]. This observation suggests that sAC might be part of a transcriptional module involved in the regulation of CRE-promoters (Fig. 3). cAMP production by nuclear sAC activates PKA, which subsequently phosphorylates CREB, altering transcription by binding to CRE sites. Sample et al. provided evidence that the scaffold protein AKAP forms a regulatory complex with PKA, potentially involving CREB and sAC [91].

Besides its effects on CRE-promoters, we were also able to show that sAC inhibition leads to impaired transcriptional activity of hormone response element (HRE) promoter activity, which is activated by aldosterone [8]. This illustrates that sAC is involved in both the cAMP/CREB and the MR pathway (Fig. 3), with a wide range of potentially regulated target genes. This is of particular interest as several studies suggested a functional interaction between aldosterone/MR and the cAMP/CREB signaling pathway [83]. One essential gene potentially regulated by sAC-dependent signaling is the serum/glucocorticoid regulated kinase-1 (SGK-1). SGK-1 is a classical MR target gene that mediates enhanced ENaC expression and therefore sodium transport [125] and is potentially stimulated by cAMP in mouse distal convoluted tubule cells [126]. sAC has also been implicated in the Rho/Rac signaling pathway, which is known to regulate cell morphology by actin modulation. Rac1 is a potent

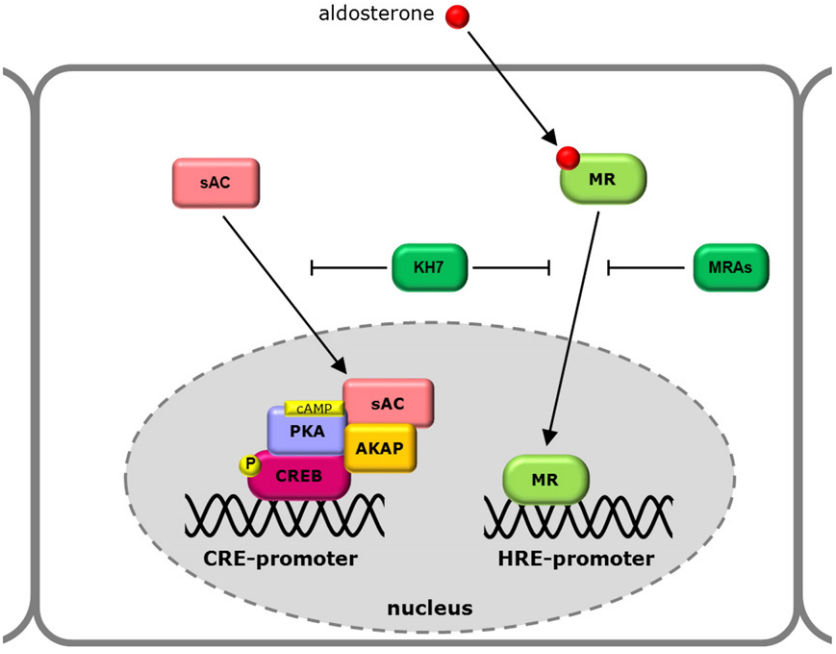

Fig. 3. Effects of sAC and MR on transcriptional regulation. Activation of the mineralocorticoid receptor (MR) by aldosterone results in nuclear MR accumulation and increased binding to hormone response elements (HRE). MR-dependent transcriptional activation can be prevented by mineralocorticoid receptor antagonists (MRAs). cAMP production by nuclear sAC activates protein kinase A (PKA), which phosphorylates the cAMP response element-binding protein (CREB). Phosphorylated CREB alters transcription by binding to cAMP responsive elements (CRE). The scaffold protein A-kinase anchor protein (AKAP) forms a regulatory complex with PKA, sAC and CREB. The sAC inhibitor KH7 impairs CRE- and HRE-dependent transcription.

activator of MR signal transduction, in that Rac1 increases MR nuclear accumulation with subsequent enhancement of MR-dependent promoter activity [127]. Notably, sAC has been described as a unique activator of the small G protein Rap1 [9]. The sAC-dependent CRE-promoter activation is also interesting in light of the finding by Krishnan et al. [128]. The group reported that PTH activates CRE-promoter transcription. Preliminary data suggest that PTH-dependent activation of CRE-promoters may be prevented by sAC inhibition (own unpublished data).

7. Conclusion

There is current evidence that sAC is a regulator of aldosterone signaling in the kidney and vascular endothelium. In renal tubular cells, aldosterone-stimulated transepithelial sodium current depends on sAC activity. In vascular endothelial cells, sAC is involved in the regulation of actin fiber composition and endothelial stiffness. sAC may be an important modulator of pathophysiological conditions in CKD and CVD patients, including endothelial dysfunction and chronic arterial stiffness. Effects of experimental sAC inhibitors such as KH7 show similarities to the effects of clinically used MRAs and are potential novel compounds for the prevention of deleterious aldosterone effects in CKD and CVD. Additional evidence from experimental and clinical studies is needed to confirm sAC as a potential therapeutic target in these common disorders.

Conflict of interest

The authors declare no conflict of interest.

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