Regulation of nephron water and electrolyte transport by adenylyl cyclases

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1Department of Medicine, University of California San Diego, La Jolla, California; 2Veterans Affairs San Diego Healthcare System, San Diego, California; 3Division of Nephrology, University of Utah Health Sciences Center, Salt Lake City, Utah; and 4Salt Lake City Veterans Affairs Medical Center, Salt Lake City, Utah

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Rieg T, Kohan DE. Regulation of nephron water and electrolyte transport by adenylyl cyclases. Am J Physiol Renal Physiol 306: F701–F709, 2014. First published January 29, 2014; doi:10.1152/ajprenal.00656.2013.—Adenylyl cyclases (AC) catalyze formation of cAMP, a critical component of G protein-coupled receptor signaling. So far, nine distinct membrane-bound AC isoforms (AC1-9) and one soluble AC (sAC) have been identified and, except for AC8, all of them are expressed in the kidney. While the role of ACs in renal cAMP formation is well established, we are just beginning to understand the function of individual AC isoforms, particularly with regard to hormonal regulation of transport and channel phosphorylation, membrane abundance, and trafficking. This review focuses on the role of different AC isoforms in regulating renal water and electrolyte transport in health as well as potential pathological implications of disordered AC isoform function. In particular, we focus on modulation of transporter and channel abundance, activity, and phosphorylation, with an emphasis on studies employing genetically modified animals. As will be described, it is now evident that specific AC isoforms can exert unique effects in the kidney that may have important implications in our understanding of normal physiology as well as disease pathogenesis.

homeostasis; parathyroid hormone; renal disease; signaling; vasopressin

Nine different membrane-bound adenylyl cyclase (AC) isoforms in mammals have been described; all of them are key enzymes in catalyzing the conversion of ATP to cAMP. The membrane-bound ACs (which is what this review refers to, unless stated otherwise) have two membrane clusters that each contain six transmembrane domains and three large cytoplasmic domains (N, C1a/b and C2a/b). C1a and C2a form the catalytic core complex; they are highly conserved and homologous to one another. The N-terminal domain varies between AC isoforms and plays a regulatory role (46).

A given cell type can express several AC isoforms, binds numerous agonists that modify cAMP production, and has a wide range of cAMP-dependent effects. This begs the question as to how cAMP is able to mediate such a variety of effects yet do this in a specific and highly regulated manner. This review focuses on one aspect of such specificity, namely, the role of unique AC isoforms in mediating particular biological actions in the kidney. However, it is important to briefly discuss the other key factors that play a role in allowing cAMP to exert so many functions, yet in a highly directed manner.

The classic paradigm was that G proteins interacted randomly with ACs; i.e., each AC molecule’s activity reflected the relative influence of potentially several G protein-coupled receptors (GPCRs). However, studies using fluorescent energy transfer-based intracellular probes indicate that AC-derived cAMP (and its downstream effectors) is confined to specific regions within the cell (3, 22). Furthermore, recent studies suggest that cAMP synthesis by specific AC isoforms can occur in vesicular compartments due to sustained activity of specific internalized receptors (3). Such association between GPCRs and ACs is likely due, at least in part, to A-kinase-anchoring proteins (AKAPs) that act as scaffolds to attached ACs to the plasma membrane or to the cytoskeleton (21, 22). These AKAPs, of which there are >50 members, maintain cAMP levels within discrete ranges immediately surrounding ACs (comparable to a small cloud) through direct binding to specific phosphodiesterases (PDEs) and dephosphorylases (such as calcineurin). Of equal importance, AKAPs also maintain localized activation of the two major cAMP effectors through direct binding to protein kinase A (PKA; as the name implies) and indirectly complexing with exchange protein directly activated by cAMP (Epac; a third cAMP target, the cAMP-gated ion channels, has been much less studied) (21, 22). PKA has long been viewed as the primary effector of cAMP effects on a variety of renal transporters and channels; however, recent studies reveal that Epac, which activates the small G proteins Rap1 and Rap2 (73), also mediates several cAMP actions in the kidney, including Ca2+ mobilization, urea transport, and other effects (45, 87, 90).

In addition to varying subcellular localizations, the nine membrane-associated ACs may be differentially regulated by Gαq and Gβγ G protein subunits, divalent cations, small molecules, and posttranslational modification. For a detailed description of individual AC regulation, the reader is referred to several excellent reviews (6, 34, 88); however, we will briefly...
summarize unique aspects of AC isoform regulation. At the outset, it is important to note that specific AC isoform inhibitors or activators, although in active development, have not yet been identified (76); most of our information about the characteristics of individual AC isoforms derives from heterologous expression systems, genetically modified mouse models, or inferences drawn from patterns of pharmacological manipulation of regulatory factors. With the exception of AC2, mice have been developed with global knockout of each of the nine membrane-bound AC isoforms (reviewed in Refs. 57 and 70). Unique phenotypes have been associated with each conventional AC isoform knockout with the exception of AC4, where no abnormal phenotype has been noted. A recent detailed and critical analysis of the literature pointed out that major gaps exist in our understanding of AC isoform properties (34); however, some generalities can be made. AC isoforms can be grouped according to response to changes in Ca\(^{2+}\); those that are directly stimulated by Ca\(^{2+}\) (AC1, AC8 and possibly AC3), directly inhibited by Ca\(^{2+}\) (AC5 and AC6) or those with no direct response to Ca\(^{2+}\) (AC2, AC4, AC7 and AC9). The AC isoforms also appear to have differential responsiveness to protein kinase C (PKC), wherein AC6 is inhibited while AC2, AC3, AC5, and AC7 are activated. Regulation of individual AC isoforms by G proteins is quite controversial and requires substantial elucidation (70).

**Soluble AC**

There is a single mammalian soluble AC (sAC) isoform (reviewed in Ref. 83). The sAC is diffusely located throughout the cytoplasm and also exists in discrete locations within various organelles. Its activity is increased by divalent cations (predominantly Ca\(^{2+}\)) and HCO\(_3\)^\(^{-}\); unlike membrane-bound ACs, it is not stimulated by forskolin. The sAC can move between subcellular compartments in response to varying acid-base status, resulting in regional increases in cAMP that can affect cellular respiration and survival (83).

**Expression of AC Isoforms Within the Nephron**

Determination of the renal region- or cell-specific pattern of AC isoform expression originally was based upon inferences drawn from the use of agents that altered intracellular Ca\(^{2+}\) signaling. If agonist-stimulated cAMP accumulation was unaffected by modifying Ca\(^{2+}\) signaling, then the Ca\(^{2+}\)-insensitive isoforms (AC2, AC4, AC7, AC9) were assumed to be involved. If increasing intracellular Ca\(^{2+}\) inhibited agonist-evoked cAMP, then AC5 or AC6 was implicated, while if this augmented cAMP levels, then AC1, AC3, or AC8 was invoked. However, such conclusions were fraught with potentially complicating factors and did not clearly identify individual AC isoforms. Subsequent studies assessed AC isoform mRNA presence; however, while the absence of a given AC isoform mRNA is generally reasonable evidence that the isoform is missing, its presence does not guarantee that the protein is expressed. Protein analysis, whether by Western blotting or immunostaining, has provided useful insights although some caution must be taken in interpretation of these studies given the difficulties in developing highly specific antibodies; all the membrane-bound ACs are structurally similar, so obtaining unique antigenicity has been challenging. In addition, only rodent kidney AC isoform expression has been explored in any detail (for a summary of the ensuing discussion, see Table 1). Given these caveats, the following describes our current understanding of AC isoform expression along the nephron.

**Proximal tubule.** In studies examining rat proximal tubule, Bek et al. (7) determined that this nephron segment expressed AC2, AC3, AC6, AC7, and AC9 mRNA. They also noted immunostaining for AC2, AC3, AC4, and AC9 in the proximal tubule, while the antibodies for the other AC isoforms were deemed unreliable. Interestingly, AC isoforms were detected only on the luminal membrane, while Western blot analysis detected only AC2 and AC9 in brush-border membranes. These finding suggested that AC isoforms may be differentially located within the proximal tubule, raising the possibility that associated signaling molecules and receptors are similarly localized; this issue requires further examination. In addition, this group detected AC7 only in the proximal tubule and not elsewhere in the nephron. Chabardes et al. (12), examining only AC4, AC5, and AC6 mRNA expression, detected only AC6 in rat proximal tubule.

**Loop of Henle.** The thin limb of Henle’s loop in rats expresses AC3, AC4, AC6, and AC9 mRNA and protein (although, as stated above, AC6 staining was felt to be uninterpretable) (7). AC6, but not AC4 or AC5, mRNA was also reported in rat thin limb (12), while AC3 immunostaining was detected in rat thin limbs (38). In the thick ascending limb (TAL), only AC6 mRNA and AC9 mRNA and protein were detected (7). Another group also reported AC6, but not AC4 or AC5, mRNA in rat TAL (12). No AC3 immunostaining was observed in mouse TAL, although it was detected in the macula densa (60). In contrast, both AC3 and AC4 mRNA and

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**Table 1. Expression of AC isoforms within the nephron in rodents**

<table>
<thead>
<tr>
<th>Region</th>
<th>AC1</th>
<th>AC2</th>
<th>AC3</th>
<th>AC4</th>
<th>AC5</th>
<th>AC6</th>
<th>AC7</th>
<th>AC8</th>
<th>AC9</th>
<th>sAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal tubule</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Thin limb</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Thick ascending limb</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Macularis</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Distal tubule</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cortical CD</td>
<td>−</td>
<td>±</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Outer medullary CD</td>
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<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<td>−</td>
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<td>Inner medullary CD</td>
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<td>±</td>
<td>−</td>
<td>+</td>
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<td>+</td>
<td>−</td>
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<tr>
<td>Principal cell</td>
<td>−</td>
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<td>−</td>
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<td>−</td>
</tr>
<tr>
<td>Interstitial cell</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

*The table represents a distillation and interpretation of the data; specific information upon which this summary is based can be found in the text. AC, adenylyl cyclase; CD, collecting duct.*
protein have been reported in rat macula densa (7). Soluble AC immunostaining has been reported in TAL in two studies (33, 54).

Distal tubule. The distal tubule in the rat contains AC2, AC3 (albeit inconsistently), AC4, AC6, and AC9 mRNA with a similar pattern of expression detected by immunostaining (7). AC3 immunostaining colocalized with the Na\(^+\)-Cl\(^-\) cotransporter (NCC) in the mouse distal convoluted tubule, and both were detected only apically (60). AC6, but not AC4 or AC5, mRNA was observed in rat and mouse distal tubule (12). Finally, sAC immunostaining was reported in the distal tubule (54).

Connecting tubule/collection duct. The collecting duct (CD) has been the most extensively evaluated region of the nephron with regard to AC isoform expression. Microdissected rat cortical CD (CCD) expressed AC2, AC4, AC5, AC6, and AC9 mRNA and protein (again, AC5 and AC6 staining could not reliably be determined) (7). AC5 and AC6, but not AC4, mRNA was observed in rat CCD (12, 36). Mouse CCD contained AC3, AC4, AC5, and AC6 mRNA (79). Immunostaining for AC3 was present in the mouse connecting segment and CCD (60). Soluble AC protein appears to be abundantly present in the CCD (33, 54, 55). The outer medullary CD (OMCD) has a similar pattern of AC isoform expression as the CCD (7, 12, 36, 79). Further analysis of these two regions of the CD reveals a differential pattern of expression between principal and intercalated cells. In particular, Pastor-Soler et al. (54) reported colocalization between sAC and the vacuolar ATPase (V-ATPase), but not aquaporin-2 (AQP2), in rat kidney, suggesting intercalated cells selectively express sAC within the kidney. In contrast, two other studies using a different antibody reported that sAC immunostaining was present in both rat principal and intercalated cells (33, 55). Paunescu et al. (55) noted that sAC and the V-ATPase colocalized apically and subapically in type A intercalated cells, while they colocalized basolaterally in type B intercalated cells. Finally, in situ hybridization studies detected AC5 only within intercalated cells based on colocalization with V-ATPase, but not AQP2 (36). Thus, while the CCD and OMCD express several AC isoforms, AC5 is uniquely found in this region of the nephron due to selective intercalated cell expression.

Studies on inner medullary CD (IMCD) AC isoforms have yielded somewhat conflicting results. Studies have consistently shown that the highest mRNA and/or protein expression was found for AC6 in rat and mouse IMCD (7, 12, 36, 38, 79, 84). Expression levels of AC6 were inversely correlated with fluid intake, pointing to a predominant role of this isoform in urinary concentration (65). AC5 expression was inconsistently found to be present, and AC3 mRNA and protein were observed in mouse IMCD by some investigators (65, 79) but not others (66). AC8 was consistently absent, and some studies found AC9 mRNA and possibly protein (7, 38, 65). AC4 mRNA was not detected in rat IMCD by one group (12); however, others have observed AC4 mRNA and/or protein in rat and mouse IMCD (38, 65, 79, 84). AC7 and AC9 mRNA, but not protein, were detected in rat and mouse IMCD (36, 65, 79), although one study in rats did find AC9 immunostaining in the IMCD (7).

Consideration of the above complex patterns of AC isoform expression in the nephron suggests that, as previously mentioned, these proteins may mediate region-specific effects. Certain isoforms (AC6 and AC9) appear to be fairly ubiquitously expressed along the nephron; while they may mediate the effects of specific agonists that are unique to a given nephron segment, such conclusions cannot be drawn from simple expression analysis. One study deserves special consideration wherein a green fluorescent protein (GFP) reporter gene was inserted into the mouse AC6 gene (18). GFP was expressed along the entire nephron, with the highest expression in the distal tubule and CD. Double labeling with a primary cilia marker indicated that, in addition to basolateral staining, the primary cilium at the luminal cell membrane was stained. Similarly, sAC appears to be primarily found in the proximal tubule, distal nephron, and particularly the CD; its role in bicarbonate sensing may explain this nephron localization (55, 83). Finally, it remains to be determined whether differences in AC isoform expression within the nephron vary between species.

Juxtaglomerular apparatus. While not part of the nephron, consideration of AC expression in juxtaglomerular cells (JG) is included, since, besides the nephron, it is the region within the kidney where the function of individual AC isoforms has been relatively intensely studied. Most data derive from studies using cultures of JG cells. Ortiz-Capisano et al. (53) determined that mouse JG cells contain AC5, but not AC6 protein. Renin secretion by JG cells has been referred to as the “Ca\(^{2+}\) paradox” since agonists that increase intracellular Ca\(^{2+}\) typically elicit endocrine factor secretion, but paradoxically inhibit JG renin release. Since renin secretion is stimulated by cAMP, the conclusion was that Ca\(^{2+}\)-inhibited AC isoforms (AC5 and/or AC6) were involved. This group observed that chelation of intracellular Ca\(^{2+}\) stimulated renin release and that this was prevented by a “selective AC5” inhibitor (NKY80). While these results support the notion that a Ca\(^{2+}\)-inhibited AC isoform is involved, the inhibitor is not highly AC5 specific. Another study noted that parathyroid hormone (PTH) increased JG cell non-Ca\(^{2+}\)-sensitive cAMP accumulation without increasing renin secretion, suggesting compartmentalized cAMP actions (4). In contrast to the hypothesis that AC5 might be the predominant isoform for renin secretion, Aldehni et al. (2) observed that under basal conditions AC5 knockout mice have similar plasma renin concentrations (PRC) compared with wild-type mice; however, AC6 knockout mice display significantly increased PRC under basal conditions. Agonist-induced increases in PRC were reduced in both AC5 and AC6 knockout mice, although the impairment was more severe in AC6 knockout mice.

Other regions of the kidney. Information on AC expression/function in other parts of the kidney is limited. To our knowledge, only two studies exist which determined the role of AC1 or AC3 in podocytes (89) and glomeruli/renal artery (59), respectively. AC1 knockout mice show an aggravated protein loss in response to albumin overload, while having no other renal phenotype. The role of AC3 in glomeruli remains elusive; however, it was speculated to initiate signals leading to paracrine regulation of renin secretion. The role of AC3 in the renal artery needs to be determined.

Physiological Effects of AC Isoforms

Role of AC isoforms in mediating forskolin- and hormone-stimulated cAMP formation. Basal renal AC activity of rats and rabbits was shown to be higher in the medulla vs. cortex (24,
effect that was absent in both wild-type and AC6 knockout mice. The latter results possibly indicate that the AC6 isoform is required for PTH-stimulated cAMP formation in the renal cortex. In contrast to findings pointing toward a significant role of AC3 in murine renal cAMP formation in this cell type.

Role of AC isoforms in modulating renal tubule electrolyte transport. Thick ascending limb. AVP stimulates Na\(^+\) transport in the TAL. Exposure to AVP increases Na\(^+\) transport in micropunctured rat and isolated mouse TAL (although there is some controversy on whether both cortical and medullary TAL [mTAL and cTAL, respectively] respond to AVP in the mouse) (20, 72). This stimulatory effect of AVP on TAL Na\(^+\) transport is mediated by modulation of the Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter (NKCC2). In isolated rat TAL, AVP increased steady-state surface NKCC2 via PKA activation but not via Epac (10). Short-term activation of NKCC2 by AVP involves phosphorylation: one study observed phosphorylation at threonine residues 96 and 101 (T96 and T101) (29), while another noted only T96 phosphorylation (32). Two additional serine phosphorylation sites of rat NKCC2, S126 and S874, have recently been identified (32); however, the role of these serine, as well as the threonine phosphorylation sites, in modulating NKCC2 activity and trafficking is incompletely understood. Rieg et al. (66) recently determined the role of AC6 in modulating NKCC2 protein expression, phosphorylation and localization. Unfortunately, despite extensive effort, the authors were unable to detect S874 NKCC2 protein in murine kidney. Under basal conditions, total renal NKCC2 expression was 50% lower in AC6 knockout compared with wild-type mice and this was associated with a 1.8-fold greater expression of pS126 NKCC2 (Fig. 1). The increased S126 phosphorylation might protect the AC6 knockout mouse from developing a more severe salt-losing phenotype (see below). Immunofluorescence labeling and confocal analysis confirmed a reduced total NKCC2 staining in kidneys of AC6 knockout mice compared with wild-type mice in both mTAL and cTAL. In the mTAL, the abundance of pS126 NKCC2 was low in wild-type mice and not detectable in AC6 knockout mice. In the cTAL, no apparent differences were observed in labeling intensity of pS126 NKCC2 between genotypes, which had overall greater labeling intensity than in the mTAL. The labeling intensity for total NKCC2 in mTAL and cTAL after AVP treatment was not different between genotypes. However, labeling of pS126 NKCC2 significantly increased in the mTAL of wild-type mice in response to AVP, a finding completely absent in AC6 knockout mice. In contrast to the mTAL, AVP did not affect labeling intensity of pS126 NKCC2 in cTAL of either genotype.

Distal tubule. The activity of the distal tubule Na\(^+\)-Cl\(^-\) cotransporter (NCC) is subject to complex regulation, including AVP stimulation of Na\(^+\) reabsorption (23) through phosphorylation at multiple sites (T53, T58, S71, and S124; rodent nomenclature) (51, 56, 69). Under basal conditions, AC6 knockout mice had a 1.9-fold higher total NCC expression compared with wild-type mice, while pT58 NCC was not different between genotypes (66). The greater NCC expression is possibly a consequence of reduced NKCC2 expression/activity, resulting in greater delivery of NaCl from the TAL.
Fig. 1. Integrated renal and blood pressure phenotype of AC6 knockout mice. AC6 may stimulate total renal Na\(^{+}\)-K\(^{+}\)-2Cl\(^{-}\) cotransporter (NKCC2) protein abundance in medullary and cortical thick ascending limb (although the latter is controversial). Increased AC6-independent phosphorylation of NKCC2 at serine 126 (S126) in the medulla might help to stabilize NKCC2 activity in the absence of AC6. Upregulation of Na\(^{+}\)-Cl\(^{-}\) cotransporter (NCC) in AC6 knockout mice may compensate for reduced NKCC2 activity. In the aldosterone-sensitive distal nephron, the expression, but not single channel activity, of the epithelial sodium channel (ENaC) is reduced in AC6 knockout mice under basal conditions, while AVP-stimulated ENaC single-channel activity is absent. Total cortical and medullary aquaporin-2 (AQP2) were not different between genotypes; however, in the medullary portion of the collecting duct, phosphorylation of AQP2 at S256 and S269 (the latter only found in the apical plasma membrane) was severely reduced. In contrast to the medulla, cortical AQP2 phosphorylation was comparable to wild-type mice, indicating that possibly a different AC isoform regulates vasopressin-mediated water transport in the cortex. Impaired renal NaCl and fluid absorption decreases blood pressure and increases plasma renin concentration. AC6 knockout mice have comparable urinary urea excretion; however, inner medullary UT-A3 protein expression is reduced. The expression of proximal tubular Na\(^{+}\)-phosphate transporters Npt2a and Npt2c are also severely reduced (unpublished observations), and AC6 knockout mice have phosphaturia (64). Possibly as a consequence of intact feedback mechanisms in AC6 knockout mice, renal resistance to vasopressin and parathyroid hormone causes both hormones to be significantly elevated. In summary, AC6 knockout mice have nephrogenic diabetes insipidus and a mild Bartter syndrome. See the text for a detailed description.

into the distal tubule. Qualitative immunohistochemistry and quantitative laser scanning confocal microscopy indicated that AVP treatment did not change the labeling intensity for total NCC in the DCT of either group. In contrast, labeling and protein expression of pT58 NCC significantly increased in response to AVP in wild-type, but not in AC6 knockout mice. The signaling pathway(s) involved in AVP-induced NCC phosphorylation are unknown, but in addition to AC6, might involve the “with-no-K[Lys] kinases”/STE20/SPS1-related proline/alanine-rich kinase/oxidative stress responsive kinase-1 pathway, which, when activated, results in increased NCC at the cell membrane and increased NCC activity (56, 71). Further analysis of AC6 knockout mice indicated 1) mild hypokalemia (66), 2) mild alkalosis (66), 3) mild urinary Na\(^{+}\)/K\(^{+}\) loss (65, 66), 4) lower blood pressure (2), and 5) elevated plasma PRC (2). Taken together, the changes found in the TAL and DCT of AC6 knockout mice possibly result in a mild salt-losing phenotype and cause clinical features consistent with Bartter syndrome (shown in Fig. 1).

COLLECTING DUCT: The CD epithelial Na\(^{+}\) channel (ENaC) is regulated by multiple factors, including by AVP-induced cAMP. Infusion of AVP in Brattleboro rats markedly increased ENaC β- and γ-subunit mRNA levels in the renal cortex (52). Similar results were obtained in Sprague-Dawley rats after either partial water restriction or AVP infusion. In isolated, perfused CCD of Brattleboro rats, AVP increased amiloride-sensitive Na\(^{+}\) transport (52). Bugaj et al. (9) demonstrated that AVP increases ENaC activity (open probability; \(P_o\)) in acutely isolated split-open CCD; furthermore, this effect was dependent on AC and PKA. Recently, mice with principal cell-specific AC6 knockout were studied (67). Under basal conditions, these mice had decreased renal cortical mRNA content of α-, β-, and γ-ENaC as well as reduced total cell protein of α- and γ-ENaC compared with wild-type mice. Whereas baseline ENaC activity in acutely isolated split-open CCD was not different between genotypes, AVP-stimulated ENaC \(P_o\), and the increase in channel number was absent in principal cell-specific AC6 knockout compared with wild-type mice. In contrast, principal cell-specific AC3 knockout mice show a comparable \(P_o\) and AVP-stimulated \(P_o\) compared with wild-type mice (43). Blood pressure in principal cell-specific AC3 knockout mice was not different from wild-type mice under normal-, low-, or high-Na\(^{+}\) intake. Taken together, these results demonstrate that AC6 is required for normal expression and function of ENaC in principal cells.

Role of AC isoforms in modulating renal tubule water transport. As discussed above, studies in rats and mice have provided evidence for AC3 and AC6 in the regulation of AVP-stimulated cAMP formation in the CD via activation of the vasopressin V2 receptor (V2R). With regard to water transport, AVP-stimulated cAMP induces sequential phosphorylation of aquaporin-2 (AQP2) at S256 (40) and S269 (39), resulting in apical plasma membrane accumulation of AQP2,
and enhanced water reabsorption provided a favorable osmolar gradient (25, 49). Studies focusing on the role of specific AC isoforms in urinary concentration are limited. So far, only clear evidence exists for AC3 and AC6, and this is based on studies in genetically modified animals; other AC isoform knockout animals have not been challenged with water loading or water deprivation, and AVP responses have not been studied. However, with free access to food and water, global AC3 knockout mice, although having a lower glomerular filtration rate, have normal urine osmolality (60). In a principal cell-specific AC3 knockout mouse, total and S269 AQP2 expression were comparable to wild-type mice (43). Global AC5 knockout mice appear to have normal urine osmolality under baseline conditions (65) (Table 2). In contrast, global AC6 knockout mice show reduced urine osmolality under basal conditions associated with greater fluid intake and greater brain AVP mRNA expression compared with wild-type mice (18, 65, 68) (Table 2). In AC6 knockout mice no evidence for compensation by other AC isoforms was detected (65). Principal cell-specific AC6 knockout mice show similar findings: lower urine osmolality compared with control mice (68). Western blotting and immunohistochemistry indicated that AC6 knockout mice have lower amounts of pS256 AQP2 compared with wild-type mice; while pS269 AQP2 was not detectable (65). AVP-induced phosphorylation of AQP2 at S269 is completely absent in AC6 knockout mice; moreover, increases in pS256 AQP2 and total AQP2 trafficking into the apical membrane are attenuated in the IMCD of AC6 knockout compared with wild-type mice. Taken together with reduced TAL NKCC2 activity, it is possible that the markedly impaired urinary concentrating ability in global AC6 knockout mice is due to a combination of effects in the CD and the TAL (Fig. 1). Finally, it should be noted that there may be differences between the medulla and cortex with regard to AVP-stimulated AC6-mediated regulation of NKCC2 and AQP2: the function of these molecules is impaired in the medulla, but perhaps not in the cortex, in the setting of AC6 deficiency. It may be that different AC isoforms modulate AVP action in the cortex and the medulla; this intriguing possibility requires further exploration.

Pathophysiology of AC Isoforms in Polycystic Kidney Disease

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most commonly inherited renal diseases, ultimately resulting in end-stage renal disease. The reader interested in the complex pathophysiological aspects of this disease is referred to excellent recent reviews (35, 81, 85, 91). There is now accumulating evidence indicating that the AVP-cAMP signaling pathway directly regulates cyst growth in ADPKD. In rat and mouse models of human ADPKD (named PCK and pcy, respectively), renal cAMP production and AQP2 protein expression are significantly increased compared with their wild-type counterparts, and treatment with the V2R antagonist OPC-31260 significantly inhibited renal cAMP formation and

Table 2. Summary of water homeostasis and renal arginine-vasopressin (AVP)-stimulated cAMP formation in genetically modified mice

<table>
<thead>
<tr>
<th></th>
<th>Water Intake, ml/day</th>
<th>Urine Osmolality, mmol/kgH2O</th>
<th>AVP-Stimulated cAMP Formation (Acutely Isolated Renal Tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>ND</td>
<td>1,501 ± 277</td>
<td>ND</td>
</tr>
<tr>
<td>Conventional AC3−/− (60)</td>
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<td>2,361 ± 412</td>
<td></td>
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<tr>
<td>WT</td>
<td>ND</td>
<td>1,988 ± 81</td>
<td>ND</td>
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<tr>
<td>Conventional AC5−/− (65)</td>
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<td>2,089 ± 53</td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>6.9 ± 0.3</td>
<td>1,460 ± 43</td>
<td>Comparable to WT</td>
</tr>
<tr>
<td>Conditional AC3−/− (43)</td>
<td>6.7 ± 0.5</td>
<td>1,392 ± 23</td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>5.0 ± 0.3</td>
<td>2,027 ± 160</td>
<td>Impaired vs. WT</td>
</tr>
<tr>
<td>Conventional AC6−/− (65)</td>
<td>13.5 ± 0.9*</td>
<td>875 ± 94*</td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>2.19 ± 0.1</td>
<td>3,721 ± 168</td>
<td>Impaired vs. WT</td>
</tr>
<tr>
<td>Conventional AC6−/− (18)</td>
<td>3.32 ± 0.4*</td>
<td>1,612 ± 62*</td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>3.6 ± 0.8</td>
<td>1,938 ± 189</td>
<td>Impaired vs. WT</td>
</tr>
<tr>
<td>Conditional AC6−/− (68)</td>
<td>4.61 ± 1.1*</td>
<td>1,428 ± 91</td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>4.62 ± 0.2</td>
<td>2,195 ± 147</td>
<td>ND</td>
</tr>
<tr>
<td>Conditional AC6−/− (unpublished observations)</td>
<td>11.0 ± 0.2*</td>
<td>952 ± 98*</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. WT, wild-type; ND, not determined; AQP2, aquaporin-2. *P < 0.05 vs. WT.
eighth either stopped disease progression, inhibited disease development, or even caused regression of the disease (28). In addition, crossing PCK with Brattleboro rats (the latter lacks endogenous AVP) resulted in reduced renal cAMP formation and greatly inhibited cystogenesis, while administration of AVP to the F1 generation restored the cystic phenotype (86). Recent studies in humans found that the V2R antagonist OPC-41061 (tolvaptan) slowed cyst growth and improved renal function (37, 80, 82). Since AC6 had been implicated in mediating AVP-stimulated cAMP formation, and since AC6 has been reported to be expressed in primary cilia (18) (defects in cilia-mediated signaling activity is a key factor that leads to cyst formation), the role of AC6 in ADPKD was evaluated (61). Mice with principal cell-specific knockout of polycystin-1 were compared with mice with principal cell-specific knockout of polycystin-1 and AC6; deficiency of AC6 markedly ameliorated cyst formation and renal injury in this PKD model, even though double knockout mice showed slightly enlarged kidneys compared with wild-type mice. A reduced activation of the B-Raf/ERK/MEK pathway, important for cell proliferation in ADPKD, might explain the reduced kidney size and improved kidney function. Notably, urinary cAMP excretion and renal cAMP content were unchanged in double mutant mice compared with CD-specific polycystin-1 knockout mice, suggesting that the specific AC isoform may be more important than the total cellular cAMP content. In the final analysis, it is noteworthy that, in the major ADPKD trial, tolvaptan administration was complicated by high adverse events related to increased aquarexis (37, 80, 82); even more problematic is the recent FDA ruling limiting tolvaptan use to <30 days due to the risk of liver injury (1). Thus the need for better therapies for ADPKD, including possible treatments aimed at AC6 or specific pathways that AC6 activates, is evident.

Perspectives and Significance

Multiple AC isoforms are expressed in renal epithelial cells, often in the same cell. While knockout mice for each of the AC isoforms have been generated, for the majority of them their exact role in modulating kidney function is not known. There is accumulating evidence for an important role of AC6 in control of renal electrolyte and water transport involving transport systems in the proximal tubule, thick ascending limb, distal tubule, and CD. The complexity of the hormone-AC interaction may be further increased by the fact that one specific receptor could couple to different AC isoforms in different nephron segments. Important issues to be clarified in future studies include the identification and regulation of transport pathways by AC under physiological and pathological conditions, e.g., acute kidney injury, chronic kidney injury, polycystic kidney disease, and others. The role of apical vs. basolateral signaling deserves further consideration, as does the differences in AC action between the medulla and cortex. The use of conventional and conditional gene knockout mice will help to decipher the biological role of specific AC isoforms and may lead to a better understanding of their role in renal physiology and pathophysiology.

ACKNOWLEDGMENTS

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ADENYLYL CYCLASE AND KIDNEY FUNCTION


