Novel role of ouabain as a cystogenic factor in autosomal dominant polycystic kidney disease

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The Na-K-ATPase plays an essential role in cell biology not only through its classic action as an ion pump but also as a signaling molecule that transduces the effects of ouabain and other cardiotonic steroids into cell responses. The discovery of this signaling function for the Na-K-ATPase took place long after its ion transporter properties were described and initiated a fascinating field of investigation that is rapidly expanding. The finding of a “non-pumping” function for the Na-K-ATPase also changed the traditional view of ouabain, from a compound that, at “toxic” concentrations, served to inhibit and define Na-K-ATPase activity, to a molecule that, at relatively low concentrations, has cell-regulatory properties. Results from a number of laboratories now show that ouabain activation of Na-K-ATPase-mediated intracellular signaling represents a novel mechanism for the modulation of a variety of cell processes involved in both normal cell physiology and disease. Ouabain has been shown to influence growth, apoptosis, and function (i.e., Na+ absorption) of the normal kidney epithelium. More recently, ouabain was found to stimulate cell proliferation and transepithelial fluid secretion by renal cyst epithelial cells cultured from patients with autosomal dominant polycystic kidney disease (ADPKD). This article reviews new advances in the field of ouabain-induced signaling function of the Na-K-ATPase, emphasizing the potential cystogenic effects of circulating levels of ouabain in the pathogenesis of ADPKD.

The Na-K-ATPase

Structure and classic ion transporter function. The Na-K-ATPase or “Na pump” is an ion transporter within the plasma membrane of most animal cells that utilizes the energy from the hydrolysis of ATP to catalyze the movement of Na+ out of the cell in exchange for K+ in a 3 Na+:2 K+ stoichiometry (27, 89, 186). Na-K-ATPase establishes transmembrane chemical gradients for Na+ and K+ that are essential for maintaining cell osmolarity, volume, and pH, resting membrane potential, and the electrochemical energy for secondary transport of ions and nutrients across the cell membrane (17, 41, 58, 72, 135, 199, 246). In the kidney, the Na-K-ATPase is expressed within the basolateral membranes of tubule epithelial cells, where it has a central role in the reabsorption and secretion of a variety of solutes. The differential expression and function of the Na-K-ATPase and its particular regulation along the nephron is key to the formation of urine with the appropriate volume and composition and for the maintenance of body salt and water homeostasis (58).
The Na-K-ATPase is composed of a heterodimer of two main polypeptides, the α- and β-subunits (Fig. 1A). The α-polypeptide constitutes the catalytic subunit of the enzyme, containing the binding sites for ATP, Na+, K+, and several regulators of the Na-K-ATPase (142). The α-polypeptide is a 10-transmembrane-spanning protein, with intracytoplasmic N and C termini, a large intracellular region, and 5 small extracellular loops (141). The ATP-dependent translocation of ions takes place through a reaction cycle characterized by Na+ and K+-induced conformational changes of the α-subunit, which oscillates between two main states, designated E1 and E2 (10). These conformational changes are driven by the alternating covalent phosphorylation and dephosphorylation of an aspartate residue located between transmembrane helices 4 and 5 of the protein and involve the sequential movement of three portions of the molecule, the actuator (A), nucleotide binding (N), and phosphorylating (P) domains (32, 201). While ATP hydrolysis and ion translocation primarily occur through the α-subunit, the Na-K-ATPase also requires the β-subunit for its activity. The β-polypeptide is a type II glycosylated protein that has an N-terminal cytoplasmic tail, a single transmembrane domain, and most of its mass facing the extracellular medium (64). The β-subunit acts as a chaperone protein that directs the correct folding and targeting of the α-polypeptide during its synthesis and delivery to the plasma membrane (63). In addition, the β-subunit serves as an adhesion molecule. In the kidney, the interaction between β-subunits from neighboring cells helps to maintain the structural integrity and apical-to-basal polarity of the tubule epithelium (37, 173, 211).

In mammals, four α-polypeptides (α1, α2, α3, and α4) and three β-subunits (β1, β2, and β3) are expressed in different combinations and in a cell type-specific and developmentally regulated manner (20, 116, 138, 196). Distinct association of α- and β-isosforms results in multiple αβ dimers or isozymes of the Na-K-ATPase. The α1- and β1-isosforms are widely expressed and present in most cells, including renal epithelial cells. In contrast, the other α- and β-isosforms have a more limited pattern of expression. For example, α2 predominates in adipocytes, muscle, the heart, and the brain, α3 is abundant in nervous tissues, and α4 is confined to the male germ cells in the testis. Similarly, the β-isosforms are distributed in a tissue-dependent manner. The β2-polypeptide is found in skeletal muscle, the pineal gland, and nervous tissues, and β3 is expressed in the testis, retina, liver, and lung (21, 138). Interestingly, the pattern of Na-K-ATPase isozyme expression is altered in some pathological states, including several cardiovascular and neurological diseases (8, 28, 39, 65, 145). Examination of tissues enriched with a specific Na-K-ATPase isozyme and analysis of various α- and β-isofrom combinations in heterologous expression systems have shown that each Na-K-ATPase isozyme has unique enzymatic properties (21, 146, 181). The α-isofrom is responsible for most of the functional dissimilarities between the Na-K-ATPase isozymes, with the β-subunit only modestly influencing the affinity of the α-subunit to Na+ (52, 84, 168). One of the greatest kinetic differences among α-isosforms is their unique sensitivity to ouabain. In rodents, α1 activity is highly resistant to ouabain inhibition, whereas α2, α3, and α4 demonstrate progressively higher sensitivity (20, 157, 195). This distinct response to ouabain has been commonly used as a tool to identify the contribution of each α-isofrom to the total Na-K-ATPase activity in a sample. In contrast, in other mammals, including humans, the α1-isofrom has a higher ouabain affinity, making it difficult to distinguish α1 from the other isoforms (43, 144, 220). The finding that Na-K-ATPase isozymes have singular enzymatic properties suggests that they play specific roles (20). The advent of transgenic technologies and studies in various pathological states suggests that the αβ1 complex is the ubiquitous Na-K-ATPase that maintains Na+ and K+ transmembrane gradients. In contrast, other Na-K-ATPase isozymes

![Diagram showing Na-K-ATPase control of cell function](http://ajprenal.physiology.org/)
are responsible for cell-specific tasks. In this manner, Na-K-ATPase isozyme heterogeneity appears to have evolved as a mechanism to serve particular cell physiological needs (45, 49, 81, 87, 118, 143, 245). In addition, alterations of the function of Na-K-ATPase isozymes may play critical roles in disease (19, 40, 97, 176, 213).

The Cardenolide Ouabain

General structure and properties. Ouabain belongs to a group of compounds, the cardiotonic steroids, which are composed of a steroidal backbone, the aglycone, or genin; a five-membered unsaturated lactone ring; and a sugar moiety, which varies depending on the type of cardenolide (15). Ouabain binds to the extracellular side of the Na-K-ATPase α-subunit, preferentially docking to the E2 phosphorylated conformation of the transporter. Ouabain interferes with the reaction cycle, inhibiting the ion transport and enzymatic activity of the Na-K-ATPase (117, 216). Because of this property, relatively high doses of ouabain have been conventionally used to specifically define Na-K-ATPase-dependent ion transport (10). Ouabain is classified as a cardenolide, one of two subfamilies of cardiotonic steroids, which include several plant derivatives (3). Ouabain is found in the African plants Strophanthus gratus and Acokanthera ouabaoi. Other cardenolides are digitalis, present in the foxglove Digitalis purpurea, and digoxin, found in Digitalis lanata. The other subfamily of cardenolides comprises the bufadienolides, which include bufalin and marinobufagenin. These cardenolides are produced in the skin of the toad, Bufo marinus.

The practical use of cardenolides and bufadienolides can be traced back centuries, much before their effects on the Na-K-ATPase were known. The toxic effects of cardiotonic steroids made them useful as poisons. In addition, utilized in limited and controlled amounts, these substances were found to have therapeutic benefits, which opened their application as herbal remedies (113). It was not until ~200 years ago that cardiotonic steroids were formally used in the clinical setting. The positive inotropic effects of nontoxic doses of cardiotonic steroids made these compounds useful for the treatment of cardiac insufficiency and conferred them their general designation as “cardiotonics” (55, 80). Because digitalis and digoxin cause positive chronotropism, they have also proven useful for the treatment of atrial fibrillation (132). Despite their application in the treatment of heart failure and arrhythmias, the use of cardiotonic steroids remained mostly empirical for quite a long time (18, 214); only recently have insights into their mode of action been obtained. Simultaneous measurements of intracellular Na+ concentrations and contractile force in cardiac fibers, along with studies of the effect of extracellular K+, showed that the “cardiotoxic” effect was caused by inhibition of the Na-K-ATPase (90, 107). Subsequent experiments established a correlation between cytosolic Na+ and Ca2+ levels and the functional association of Na-K-ATPase with the Na/Ca exchanger (NCX) in cardiac cells (107, 135, 187, 219, 221, 227). A model developed for the mechanism of action of cardiotonic steroids, by which inhibition of Na-K-ATPase increases intracellular Na+ and produces a secondary rise in Ca2+ by reducing the inward movement of Na+ through the sarcolemmal NCX. The higher intracellular Ca2+ is taken up by the sarcoplasmic reticulum via the Ca-ATPase, allowing stronger con-tractility of the heart muscle when Ca2+ is released back into the cytoplasm (Fig. 1A) (24, 25). Later evidence indicated that the cardiotoxic effects of ouabain are mediated by the Na-K-ATPase α2-isofrom in mouse hearts (45, 48). Through mechanisms similar to those of the heart, ouabain can enhance contraction of vascular smooth muscle cells to augment vascular tone and produce arterial constriction in rodents (26, 49).

Oubain as an endogenous compound in mammals. Originally, ouabain and other cardiotonic steroids were thought to only be the products of plants and amphibians; however, it was later discovered that they are also endogenously expressed in mammals. This finding resulted from the search for a circulating substance that could explain Na+-dependent changes in blood pressure. The factor that was discovered to be increased in plasma after salt-induced volume expansion had the ability to partially inhibit Na-K-ATPase activity and induced natriuresis. This natriuretic factor also raised blood pressure, an effect that was later found to be dependent on the ability of the substance to block Na-K-ATPase activity within the smooth muscle of the arterial walls (60). Several different endogenous chemical scaffolds with the ability to inhibit the Na-K-ATPase were subsequently reported, and cardiotonic steroids appeared as principal candidates that fulfilled the observed properties. However, there was concern that cardiotonic steroids found in the circulation were simply contaminants from ingesting plant products in the diet. In addition, the scarce amounts and the technical difficulties in both purification and identification of these substances represented major obstacles before the existence of cardiotonic steroids as endogenous products of higher animals could be validated. Ouabain was purified from extracts of bovine adrenal gland, hypothalamus, hypophysis, and adrenal gland tumors, demonstrating the presence of this cardenolide in mammalian tissues (14, 33, 46, 88, 150, 178, 185). Ouabain was also isolated from conditioned media of PC12 and rat adrenal gland cell cultures, confirming that mammalian cells produce ouabain (100).

Hamlyn and coworkers (74) were the first to identify ouabain as a circulating substance in human plasma. Endogenous ouabain has also been found in the plasma of other species where concentrations vary in the picomolar-to-nanomolar range. Several lines of evidence suggest that ouabain functions as a natriuretic factor. Administration of anti-ouabain antibodies to rats reduced Na+ excretion by the kidney (151), and circulating levels of ouabain increased in rats after salt loading (233). In Dahl salt-sensitive rats, an intraperitoneal Na+ load stimulated the release of a ouabain-like compound into the circulation and caused an increase in renal Na+ excretion, suggesting the role of ouabain as a regulatory mechanism for the maintenance of Na+ homeostasis (57). In cultured LLC-PK1 cells, a renal epithelial cell line, ouabain inhibited apical-to-basolateral transepithelial flux of 22Na+ across the cell monolayers by decreasing the expression of Na-K-ATPase and Na/H exchanger 3 (NHE3) at the plasma membrane. This effect was mediated by a combination of endocytosis and reduced gene transcription of the ion transporters (121, 239). More recently, transgenic mice that express a mutated renal Na-K-ATPase α1-isofrom that has a greater sensitivity to ouabain exhibited a greater natriuretic response to Na+ load than wild-type mice (126).

While research has mainly advanced our understanding of the endogenous nature and effects of ouabain in different cells
and organs, relatively little is known regarding the endocrine aspects of this hormone. The site of ouabain synthesis has been mapped to the zona glomerulosa and fasciculata of the adrenal gland cortex (104, 133). The biosynthetic pathway for ouabain requires hydroxycholesterol, pregnenolone, and progesterone as precursors and follows metabolic reactions that are, in part, shared with those of other adrenal steroidal hormones (75, 179). Also, there is evidence that, once synthesized, ouabain is released into the circulation in a regulated fashion. Several conditions are associated with an increase in ouabain, including chronic renal insufficiency (191), experimental uremia (78), congestive heart failure (129, 130), hypertension (79), primary hyperaldosteronism (175), physical exercise (9), hypoxia (174), and chronic salt intake (23). Ouabain secretion is also stimulated by ACTH, angiotensin II (ANG II), and α1-adrenergic receptor agonists (179). Data on other characteristics of endogenous ouabain, such as its half-life in plasma, metabolism, and elimination, are scarce and mostly limited to the pharmacokinetic and pharmacodynamic information reported for exogenously administered ouabain (92, 169, 182, 183).

Ouabain-Na-K-ATPase Signaling

The Na-K-ATPase is a receptor and signal transducer. The detection of low levels of ouabain in the circulation encouraged studies of the nontoxic effects of cardenolides. Ouabain, in concentrations that do not completely inhibit the Na-K-ATPase, is capable of eliciting a wide variety of effects, including cell proliferation, hypertrophy, apoptosis, and changes in cell motility and metabolism. These effects are cell type specific, resulting in different functional outcomes at the tissue and organ level (14, 180, 185). A breakthrough in the mechanisms of action of ouabain was the discovery that ouabain not only affected ion transport activity of the Na-K-ATPase but also activated a series of intracellular messengers and signaling events in cells. This revealed an unforeseen novel receptor and signal transduction function for the Na-K-ATPase, which upon binding ouabain, at endogenous concentrations, mediates cellular effects (165, 229, 230). Pioneering research by Askari (99) and Xie (120) in myocardial and kidney epithelial cells showed that ouabain induces the interaction of the Na-K-ATPase with signaling proteins, leading to activation of the tyrosine kinase Src and the phosphorylation of downstream effector proteins. Allen (2, 13) also contributed to the original findings for a role of the Na-K-ATPase as a transducer of ouabain’s effects, providing the first evidence of Na-K-ATPase-mediated intracellular signaling in vascular smooth muscle cells. Additional studies extended these observations to other cell types (4, 31, 47, 53, 94, 101, 102, 140, 147, 210, 232). It is now clear that Na-K-ATPase functions as a molecular scaffold that assembles a multiprotein signaling complex known as the Na-K-ATPase signalosome (230) (Fig. 1B). Proteins found to be associated with the ouabain-bound Na-K-ATPase complex include adducin phosphoinositide-3-kinase, ankyrin, annexin 2, E-cadherin, and caveolin (113, 123). The observation that caveolin 1 also interacts with the Na-K-ATPase suggests that the Na-K-ATPase signalosome resides in caveolae (119, 122), distinctive flask-shaped invaginations of the plasma membrane rich in cholesterol (162). Indeed, cholesterol was required for ouabain-induced Na-K-ATPase signaling, and the absence of caveolin 1 abolished ouabain’s effects, confirming a role of caveolae in this signaling complex (119, 124, 172, 218). Using a clever approach, consisting of stepwise downregulation of the Na-K-ATPase in LLC-PK1 cells, Xie and associates (115, 124) showed that not all of the Na-K-ATPase at the cell surface was involved in cell signaling, but rather only the subpopulation of Na-K-ATPase associated with caveolae responded to ouabain stimulation. Therefore, the idea of two pools of Na-K-ATPase, one involved in signaling and another devoted to ion pumping, emerged.

Na-K-ATPase signaling pathway. A central event in ouabain-Na-K-ATPase signaling is tyrosine phosphorylation of a series of proteins (Fig. 1B). The Na-K-ATPase has no inherent tyrosine kinase or phosphatase activity and therefore depends on the recruitment and activation of Src. In this manner, the Na-K-ATPase serves as the ouabain binding receptor within the signaling apparatus, and Src functions as the transduction element involved in protein phosphorylation and signal amplification (165). In several cell types, including myocardiocytes, ouabain causes a dose-dependent rapid activation of Src, which is dependent on autophosphorylation of tyr 418, leading to subsequent tyrosine phosphorylation of proteins (113). Coimmunoprecipitation and coimmunoprecipitation experiments and fluorescence resonance energy transfer analysis showed that Na-K-ATPase and Src physically interact (114, 204). This association involves two protein-protein interacting sites, which include the second and third cytoplasmic regions in the Na-K-ATPase α-subunit and two sites in Src, one of which includes the kinase domain of Src. Under basal conditions, Na-K-ATPase-Src association keeps Src in an inactive state; however, ouabain binding disrupts Na-K-ATPase/Src interaction specifically at the Src kinase domain, activating the kinase (243). Interestingly, Na-K-ATPase regulation of Src activity depends on changes in the protein conformational state of the α-subunit. Induction of conformational changes in Na-K-ATPase by chemical modifiers and manipulation of the extracellular K+ concentrations showed that in E2 conformation the Na-K-ATPase releases from the kinase domain of Src, leading to Src activation (243). These experiments concluded that Na-K-ATPase signaling depends on the ability of ouabain to lock the Na-K-ATPase in the E2 conformation, thus providing, for the first time, a mechanistic view of ouabain’s action on Na-K-ATPase. Furthermore, it was later found that Na-K-ATPase signaling could be influenced by ion-induced conformational changes in the enzyme and conditions that favor transition to the E2 conformation (243).

Other events in Na-K-ATPase signaling are transactivation and phosphorylation of the epidermal growth factor receptor (EGFR) and Src-dependent assembly of the adaptor proteins Shc and Grb2 (73). This, in turn, stimulates Ras and the mitogen-activated protein kinase ERK, an important pathway involved in cell proliferation (99) (Fig. 1B). Studies in different cell types have expanded the list of pathways activated by ouabain. Additional mediators that operate downstream of the Na-K-ATPase/Src complex include phospholipase C and several isoforms of PKC, phosphatidylinositol 3-kinase (PI-3K) and AKT. Ouabain can also exert effects through the generation of reactive oxygen species from mitochondria (56, 231). Aperia and collaborators (12, 137) showed that, in primary cultures of renal proximal tubular cells, nanomolar amounts of ouabain generated spatial and temporal changes in intracellular...
Ca\(^{2+}\), which exhibited a characteristic low-frequency oscillatory pattern, involved in cell proliferation. As shown in Fig. 1B, the mechanism responsible for these Ca\(^{2+}\) changes involves the interaction of the N-terminal portions of the Na-K-ATPase \(\alpha\)-subunit and the 1,4,5-triphosphate receptor (IP3R) (11). The close proximity between Na-K-ATPase/Src and IP3R constitutes a Ca\(^{2+}\)-signaling microdomain that can transduce the effect of ouabain into endoplasmic reticulum Ca\(^{2+}\) release (125, 137, 205). The Ca\(^{2+}\) oscillations are important to enhance the activity of the transcription factor NF-\(\kappa\)B, which in turn upregulates early and late response genes (5, 12). Ouabain also increases capacitative Ca\(^{2+}\) entry in kidney proximal tubule cells via store-operated Ca\(^{2+}\) channels, which activates Akt (protein kinase B) (95). In this manner, Na-K-ATPase is able to control intracellular Ca\(^{2+}\) levels and functions as an ion transporter and a signaling molecule is summarized in Fig. 1B.

**Cellular effects of ouabain in kidney cells.** In the kidney, the most prominent consequences of ouabain’s effect are stimulation of cell proliferation and inhibition of apoptosis. Ouabain has been shown to have a mitogenic effect on opossum and rat kidney proximal tubular cells (47, 95), it protects renal epithelial cells from apoptosis induced by serum deprivation and rescues renal development from the adverse effects of malnutrition (11, 93, 110, 111). Ouabain has also been shown to have effects on renal function, including the regulation of Na\(^{+}\) reabsorption by tubular epithelial cells (22, 35, 151), modulation of epithelial cell-cell adhesion and attachment (42, 106), epithelial ciliogenesis (105), and changes in the contractile state and resistance of isolated descending vasa recta (36). Altogether, these examples show that the actions of ouabain are complex and highlight the important role that this poorly understood hormone plays in the modulation of renal development and function.

**ADPKD**

**Etiology and characteristics.** ADPKD is the most common, potentially lethal renal disorder, with a prevalence of 1 in 500–1,000 births worldwide (reviewed in Ref. 67). While the prominent feature and major cause of morbidity in ADPKD is the formation and progressive enlargement of innumerable fluid-filled cysts within the kidneys, there are also extrarenal manifestations, such as cysts in the pancreas, liver, and intestines, aortic and intracranial aneurysms, and cardiac valve prolapse (167). It is currently thought that the majority of the cysts form in utero and expand at a rate of \(~12\%\)/yr after birth (70), compressing and distorting the renal parenchyma and enlarging the kidney as much as four- to eightfold. Glomerular filtration rate in these patients remains relatively conserved for many years due to compensation by the unaffected nephrons (69). Eventually, approximately one-half of ADPKD patients progress to chronic renal failure by 50–60 yr of age, requiring kidney replacement therapy. ADPKD is the fourth most frequent cause of end-stage renal disease (ESRD), being responsible for \(~8–10\%) of all ESRD cases (6).

ADPKD is caused by mutations in either *Pkd1* and *Pkd2*, which encode for polycystin 1 and 2 (PC1 and PC2), respectively (1, 77, 96, 164). Approximately 85% of ADPKD cases are due to defects in PC1, while the remaining 15% of cases are caused by mutations in PC2. PC1 is a large glycoprotein of 4,303 amino acids and \(~450\) kDa that is expressed in epithelial cells during renal development, but its expression declines to very low levels in the adult kidney. PC1 is composed of a large N-terminal extracellular region that has adhesion and protein-protein interaction domains, 11 transmembrane-spanning domains, and a short C-terminal portion that contains a G protein binding motif (44, 155, 161). PC2 is a 968-amino acid protein of \(~110\) kDa that contains 6 membrane-spanning domains and cytosolic N- and C-terminal regions. PC2 shares homology to the transient receptor potential (TRP) family of cation channels and functions as a Ca\(^{2+}\)-permeable nonselective cation channel (66, 76, 212). PC1 and PC2 interact with each other through a coiled-coiled domain at their C-terminal region and are thought to form a macromolecular receptor/signaling complex that controls cytoplasmic levels of Ca\(^{2+}\) (170).

While the inherited mutated *Pkd* allele is present in all cells, cysts develop only in a few nephrons from clonal growth of single cells within the renal tubular epithelium. Therefore, inheritance of a mutated allele from a parent, although necessary, does not appear sufficient to induce cyst formation. The initiation of cyst formation is thought to occur either due to a somatic inactivation of the other allele of the *PKD* gene, referred to a second-hit hypothesis, or insufficient expression of the normal allele below a critical threshold, referred to as haploinsufficiency (103, 171). There is evidence for both mechanisms in cyst formation in animal models of polycystic kidney disease (PKD) (29, 83, 103, 171, 228). There is a high degree of variability in renal cyst progression even among family members that carry the same PKD mutation, suggesting that nongenetic factors influence the course of the disease. Current research is focused on identifying key factors and downstream signaling pathways that contribute to the relentless growth of renal cysts. Among these are endogenous circulating hormones and exogenous pharmacological agents, which accelerate cyst epithelial cell proliferation and/or stimulate transcellular fluid secretion. These compounds include caffeine, forskolin, vasopressin, EGF, prostaglandins, IGF, and catecholamines (reviewed in Ref. 215).

**Cellular mechanisms of cyst growth.** The development of in vitro and in vivo models of ADPKD and the use of genetic, biochemical, cell biology, and molecular biology approaches have immensely broadened our understanding of ADPKD. While the genetic basis of ADPKD has been identified, the relationship between the lack of polycystin function and the mechanisms leading to cystogenesis remains unclear. ADPKD has a complex and multifactorial pathophysiology, with several mechanisms converging to induce the formation of renal cysts. Cystic epithelial cells are characterized as being incompletely differentiated, and, while the initial cellular event initiating cystogenesis remains uncertain, it is clear that a primary manifestation is abnormal cell proliferation (68). Uncontrolled cell growth causes focal expansions of the tubule epithelium into “blister like” structures that eventually pinch off to form isolated structures that continue to expand in size. Once an isolated cyst is formed, its enlargement is determined by the combined effects of cell proliferation and the accumulation of fluid within the cyst cavity due to Cl\(^{-}\)-dependent fluid secretion (71, 202, 215). As cysts expand, there is remodeling of the
extracellular matrix (ECM) (50), excessive deposition of ECM molecules (223), inflammatory changes (136, 149), and renal interstitial fibrosis (156). A diagram of the genetic abnormality that causes ADPKD, the pathophysiological mechanisms, and the nongenetic factors that contribute to disease progression are depicted in Fig. 2.

Several signaling pathways have been implicated in PKD pathogenesis; however, cAMP plays a central role in cyst growth by stimulating both cell proliferation and transepithelial fluid secretion (16, 215, 237). Renal levels of cAMP are elevated in several rodent models of PKD, in part, due to elevated circulating levels of AVP and persistent activation of AVP V2 receptors and G protein-coupled receptors that stimulate cAMP synthesis in renal epithelial cells (62, 166, 215, 235). In ADPKD cells, cAMP stimulates PKA, which phosphorylates and activates B-Raf, a key kinase upstream of the MEK/ERK signaling pathway, leading to cell proliferation (236). In striking contrast, B-Raf is repressed in normal renal cells and cAMP does not stimulate ERK and cell growth. This unique phenotypic difference in the mitogenic response to cAMP that occurs when both PKD1 alleles become dysfunctional in normal cells appears to depend on the levels of intracellular Ca\(^{2+}\). In normal renal cells, B-Raf is maintained in an inactivated state (236). ADPKD cells have reduced intracellular Ca\(^{2+}\) levels, compared with normal human kidney cells, leading to derepression of B-Raf and cAMP activation of B-Raf/MEK/ERK signaling and cell proliferation (234).

**Na-K-ATPase in ADPKD**

Role of Na-K-ATPase ion transport in the physiopathology of renal cysts. The extraordinary appearance of an end-stage ADPKD kidney is due to the accumulation of fluid within the hundreds or thousands of cysts that grossly expand the total kidney volume. Accumulation of fluid within the cysts of ADPKD kidneys is driven by cAMP-dependent transepithelial Cl\(^{-}\) secretion (194). As with other secretory epithelia, Cl\(^{-}\)-dependent fluid secretion is dependent on the expression of key ion transporters within the apical and basolateral membranes. The Na-K-ATPase, acting in concert with K\(^{+}\) channels in the basolateral membrane, establishes and maintains the chemical and electrical gradients for secondary active transport. Chloride enters the cell through basolateral NKCC1, an electrically neutral Na\(^{+}\)-K\(^{+}\)-2Cl\(^{-}\) cotransporter, that brings Na\(^{+}\), K\(^{+}\), and Cl\(^{-}\) into the cell using the Na\(^{+}\) gradient. Thus intracellular Cl\(^{-}\) is maintained above its electrochemical gradient and is poised for rapid Cl\(^{-}\) efflux across the luminal membrane upon cAMP activation of CFTR. High concentrations of ouabain that completely inhibit Na-K-ATPase activity block cAMP-dependent net fluid secretion by intact cysts and anion secretion by polarized ADPKD cell monolayers. Because of the essential role of the Na-K-ATPase in salt and water movement across the renal epithelium, it is not surprising that this ion transporter had been the focus of early research on fluid secretion by the ADPKD cysts. These studies examined expression levels, lo-

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**Fig. 2.** Evolution of autosomal dominant polycystic kidney disease (ADPKD) cysts from epithelial cells of the renal tubules. Polycystic kidney (left), result from the growth of multiple fluid-filled cysts that originate in the renal tubules (middle) and continue developing through different pathophysiological mechanisms (right). Cysts evolve from individual cells carrying a germ-line mutation in one of the alleles for the Pkd1 or Pkd2 genes (right). Every cell in the tubule carries a mutated allele, and a somatic mutation or haploinsufficiency of the second allele is believed to trigger the disease (cells marked with stars). Initially, cell proliferation leads to the development of blister-like structures. Several abnormalities, including cell cilia malfunction, alteration in planar cell polarity, and remodeling of the extracellular matrix are also implicated in ADPKD cystogenesis. Once the cysts separate from the nephron that originated them, they continue growing through cell proliferation and transepithelial fluid secretion into the expanding cyst cavity. In addition to the genetic abnormality, nongenetic factors, such as several circulating agents, aid to enhance cyst growth and determine the progression of the disease.
calization, and activity of the Na-K-ATPase in normal and cystic kidneys of human tissues and various animal models. Normally, the Na pump displays a highly polarized distribution in renal epithelial cells, being confined to the basolateral membrane of renal epithelial tubular cells, where it is held in place by its anchoring to the cytoskeletal proteins ankyrin and fodrin (51). Due to its basolateral localization, the Na-K-ATPase pumps Na\(^+\) from the cells into the blood side of the epithelium, creating the Na\(^+\) transmembrane gradient, responsible for Na\(^+\) reabsorption through Na\(^+\) channels and transporters in the apical aspect of the cells (10). Wilson and associates (224) reported that the Na-K-ATPase was mislocalized to the apical plasma membrane of cystic epithelial cells and proposed that the active transport of Na\(^+\) by the Na-K-ATPase was responsible for the vectorial transport of NaCl and water into the lumen of the cysts.

The Na-K-ATPase mislocalization model to account for fluid secretion by ADPKD cysts was provocative, and several research laboratories set out to confirm this novel observation. However, studies in human tissues and animal models of PKD, confirmed an orthodox localization of the Na-K-ATPase in the basolateral membrane (30, 54, 86, 91, 177, 200, 203). Forskolin, a cAMP agonist, stimulated fluid secretion into intact, excised cysts from ADPKD kidneys (242). Forskolin also stimulated a lumen-negative transepithelial voltage and a positive short-circuit current in ADPKD cell monolayers mounted in Ussing chambers, consistent with active anion secretion (128). These effects of forskolin were consistent to concentrations of ouabain that completely inhibit the Na-K-ATPase when applied exclusively to the basolateral, but not apical surface of the cell monolayer, confirming normal localization of the Na pump (192). Further experiments exploring the mechanism for fluid secretion, revealed that Cl\(^-\) was the anion secreted by the ADPKD cells and that Cl\(^-\) secretion involved the Na-K-2Cl cotransporter NKCC1, K\(^+\) channels, and the Na-K-ATPase in the basolateral membrane; and the cystic fibrosis transmembrane conductance regulator (CFTR) Cl\(^-\) channel in the apical membrane (194). Based on these studies, a model was developed for Cl\(^-\) dependent fluid secretion by renal cysts, in which basolateral Na-K-ATPase generates the Na\(^+\) gradient which causes Cl\(^-\) entry across the basolateral membrane via NKCC1. Na\(^+\) and K\(^+\) that enter the cell are transported back out of the cell by the Na-K-ATPase and K\(^+\) channels, respectively. These basolateral transporters and channels maintain Cl\(^-\) above its electrochemical gradient. In the presence of cAMP, Cl\(^-\) exits the cell into the cyst lumen via the apical CFTR Cl\(^-\) channels. The efflux of Cl\(^-\) depolarizes the apical membrane, while the efflux of K\(^+\) hyperpolarizes the basal membrane. These effects generate a lumen negative transepithelial potential that drives passive movement of Na\(^+\) into the cyst lumen via the paracellular pathway (193). The net addition of NaCl to the lumen drives the osmotic movement of water into the cyst.

**Na-K-ATPase signaling in ouabain-induced ADPKD cell proliferation.** In ADPKD, ouabain at relatively high (millimolar) concentrations had been used experimentally to inhibit Na-K-ATPase activity to investigate its involvement in ion transport; however, the effects of physiological (nanomolar) concentrations remained unknown. Using primary cultures of cells isolated from renal cysts of ADPKD patients, we found that ouabain at concentrations as low as 0.1 nM enhanced cyst epithelial cell proliferation (Fig. 3A). Higher concentrations of ouabain, 0.1 μM and above, inhibited proliferation of both normal and cystic cells, likely due to the toxic effects of Na-K-ATPase inhibition (154). The proliferative response to nanomolar concentrations of ouabain in ADPKD cells agrees with the mitogenic effect of this cardiotonic steroid in other cell types, including normal rat and opossum kidney proximal tubule cells (47, 94). In contrast to ADPKD cells, nanomolar concentrations of ouabain had little effect on the proliferation of normal human kidney (NHK) cells (Fig. 3A). It remains unclear why NHK cells are unresponsive to ouabain. This discrepancy may be due to species differences or differences between immortalized and primary cells. Regardless, these studies uncovered a novel mitogenic response of ADPKD cells to physiological concentrations of ouabain. These results were one of the first reports to demonstrate that ouabain enhances cell proliferation in a hyperplastic disorder.

The effect of ouabain in ADPKD cell proliferation only takes place when the cardenolide is applied to the basolateral aspect of the cells. This agrees with our immunocytochemical studies which show normal localization of the Na-K-ATPase in ADPKD cells. In addition, we did not detect expression of the Na-K-ATPase β2-isofrom in ADPKD cells (154), an event that was suggested to be responsible for the mistargeting of the Na-K-ATPase complex to the apical membrane of ADPKD cells (222). Therefore, ouabain-induced Na-K-ATPase signaling takes place through the orthodox distribution of the Na-K-ATPase in the epithelium, which agrees with the presence of circulating endogenous ouabain at the interstitial side of the cells.

To determine ouabain’s mechanisms of action, the signaling events triggered by physiological concentrations of this cardenolide in ADPKD cells were studied (153). Ouabain-induced proliferation of cystic cells was found to be dependent on the presence and normal function of the microdomains of cholesterol-containing lipid rafts of the plasma membrane caveolae. Experimental approaches commonly used to disrupt the caveolae, such as cholesterol depletion (methyl-β-CD) and the subsequent recovery of caveolae by cholesterol replenishment, clearly modulated ouabain signaling. Moreover, the involvement of caveolae in Na-K-ATPase signaling was confirmed by the capacity of ouabain to induce, in a methyl-β-CD-sensitive manner, the phosphorylation of caveolin-1 (153), a major protein present in caveolae (124). Another early event in the ouabain-dependent Na-K-ATPase signaling in ADPKD cells is the activation of the kinase Src, through phosphorylation of Tyr 418. The activation of Src by ouabain was confirmed through specific inhibition by 4-amino-5-(4-chlorophenyl)-7-(t-butyl)pyrazolo[3,4-d]pyrimidine, or (PP2), a Src inhibitor. Moreover, ouabain activation apparently does not extend to other kinases of the Src family, since phosphorylation of Fyn kinase was unchanged by ouabain. Also, ouabain increased the phosphorylation of EGFR in ADPKD cells and the association of EGFR with the Na-K-ATPase α1-subunit (153). Ouabain increased the phosphorylation and activity of B-Raf and elevated the levels of phosphorylated ERK in ADPKD cells (153). Therefore, it is possible that the exacerbated effects of ouabain in ADPKD cells may depend on stimulation of the B-Raf/MEK/ERK pathway independently of cAMP. Interestingly, both ouabain and cAMP impinge on B-Raf, leading to ADPKD cell proliferation. The ouabain-dependent phosphorylation of...
ERK was susceptible to tyrphostin AG1478 and PP2, supporting the involvement of EGFR and Src as upstream mediators of the ouabain response in ADPKD cells. Phosphorylated ERK translocates to the nuclei of ADPKD cells, leading to transcription of genes involved in cell proliferation. There is a reduction in the expression levels of two fundamental cyclin kinase inhibitors, p21 and p27, which normally suppress cyclin-dependent kinases and the G1-to-M transition of the cell cycle. A scheme of the cellular intermediates involved in ouabain activation of cell proliferation is shown in Fig. 3B.

**Na-K-ATPase signaling in ouabain-enhanced cAMP-dependent Cl⁻ secretion in ADPKD cells.** In addition to cell growth, the effect of physiological concentrations of ouabain was explored on the transepithelial fluid secretion by polarized ADPKD cell monolayers grown on permeable supports. Ouabain had no effect when applied alone; however, it significantly enhanced cAMP-induced fluid secretion (Fig. 4A). By contrast, ouabain had no effect on fluid secretion by NHK cell monolayers. These results suggest that ouabain acts as a cofactor enhancing the effects of cAMP on fluid secretion by ADPKD cystic epithelial cells (85). Similar to the proliferative response to ouabain by ADPKD cells, ouabain only enhanced fluid secretion when applied to the basolateral side of the cells. The effect of ouabain in ADPKD cell fluid secretion was prevented by inhibitors of EGFR, Src and MEK, suggesting that these messengers are involved in the response (Fig. 4B).

To find mechanisms underlying the effects of ouabain on fluid secretion, short-circuit current (\(I_{sc}\)) was measured across ADPKD cell monolayers mounted in Ussing chambers. Ouabain enhanced the positive \(I_{sc}\) response of ADPKD monolayers to forskolin, and this current was inhibited by the CFTR inhibitor CFTR(inh)-172, consistent with an increase in cAMP-dependent Cl⁻ secretion (166). While the cellular mechanism by which ouabain enhances Cl⁻ secretion by ADPKD cells remains unclear, it is possible that the increase is due to stimulation of CFTR channel activity and not dependent on changes in the expression of either CFTR or NKCC1 (85). The capacity for ouabain to influence apical ion transport in renal cells has previously been demonstrated. In LLC-PK1 cells, ouabain was shown to regulate the function of the Na⁺/H⁺ exchanger (NHE3) via internalization and degradation of the transporter (35, 240). Additional studies are needed to delineate the effect of ouabain on the Cl⁻ secretory pathway of ADPKD cells. Figure 4B depicts the proposed mechanism for the effect of ouabain to enhance cAMP-dependent Cl⁻ secretion in ADPKD cells. In addition to ouabain’s effect on Cl⁻ secretion, it is also possible that physiological concentrations of ouabain partially inhibit the activity of the Na⁺-K⁺-ATPase in ADPKD cells, thus decreasing both the electrical and chemical driving force for Na⁺ entry via apical ENaC. Considering that the entry mechanism for Cl⁻ via the basolateral NKCC1 is electroneutral, we speculate that the secretory mechanism may be less sensitive to a small degree of inhibition in pump activity. Therefore, ouabain may decrease Na⁺-dependent fluid absorption and increase Cl⁻-dependent fluid secretion, both of which would favor net fluid secretion and exacerbate the ADPKD cystic phenotype (Fig. 4B). Undoubtedly, additional studies will be required to ascertain this possibility.

In accordance with its effect on cell mitosis and fluid secretion, ouabain enhanced forskolin-dependent expansion of microscopic cysts of ADPKD cells cultured within a three-dimensional organotypic culture system. The expansion of cysts was dose-dependently enhanced by ouabain concentrations as low as 0.1 nM and was completely blocked by the Na⁺-K⁺-ATPase inhibitor ouabain. This ouabain sensitization of forskolin-induced expansion of cysts was reversed by inhibitors of EGFR, Src and MEK, suggesting that these messengers are involved in the response. Furthermore, ouabain-induced expansion of cysts was prevented by inhibitors of PKC and PKA, consistent with the involvement of these kinases in the response. The ouabain-induced expansion of cysts was also prevented by inhibitors of ERK, PI3K and Akt, consistent with the involvement of these signaling pathways in the response. Additionally, ouabain-induced expansion of cysts was prevented by inhibitors of NFκB and STAT3, consistent with the involvement of these transcription factors in the response.

**Fig. 3.** Ouabain stimulates proliferation of ADPKD cells. **A:** physiological concentrations of ouabain stimulate the growth of human renal epithelial ADPKD cells but has only a slight effect on normal human kidney epithelial cells (NHK). Values are expressed as percentage of untreated controls, without ouabain. Modified from Ref. 152. **B:** pathway by which ouabain enhances cell proliferation and fluid secretion in ADPKD cells. Levels of ouabain far below those needed to completely block Na-K-ATPase pumping, bind to the basolaterally located Na-K-ATPase of ADPKD cells and triggers the Na-K-ATPase signaling machinery in the cell caveolae. Ouabain binding induces association of the Na-K-ATPase to EGFR and the kinase Src. Activation of Src initiates signaling machinery in the cell caveolae. Ouabain binding induces association of the Na-K-ATPase to EGFR and the kinase Src. Activation of Src initiates signaling machinery in the cell caveolae. Ouabain binding induces association of the Na-K-ATPase to EGFR and the kinase Src. Activation of Src initiates signaling machinery in the cell caveolae. Ouabain binding induces association of the Na-K-ATPase to EGFR and the kinase Src. Activation of Src initiates signaling machinery in the cell caveolae.
Ouabain affinity in ADPKD and normal human kidney cells. The molecular basis for the difference in the ouabain response between ADPKD and normal cells remains unclear; however, it may be due to a higher affinity of the Na-K-ATPase to ouabain in ADPKD cells compared with NHK cells. In kinetic assays for Na-K-ATPase activity, we found that ADPKD cells had a heterogeneous response to ouabain, consistent with two Na-K-ATPase populations with different affinities for ouabain. Approximately 20% of the total enzyme of ADPKD cells had sensitivity to ouabain that was three orders of magnitude higher than that of NHK cells (154). This characteristic property of cystic cells suggests that they have a greater capacity to bind the steroid, making them more susceptible to endogenous circulating levels of ouabain (154). The unique ouabain sensitivity found in ADPKD cells could depend on the aberrant expression of Na-K-ATPase isoforms. However, we determined that this is not the case, since only the α1β1-isozyme could be detected in both ADPKD and NHK cells (154). Alternatively, ouabain affinity may depend on an interaction between Na-K-ATPase and other proteins. The large intracellular region of the Na-K-ATPase located between transmembrane domains 4 and 5 has been shown to interact with the C terminus of polycystin 1 (159, 244). Interestingly, we found that, in insect cells exogenous expression of a construct containing the transmembrane and C-terminal domains of polycystin 1 increased the sensitivity of the Na-K-ATPase to ouabain, with an inhibition constant that was similar to that found in ADPKD cells (21). However, the same change in ouabain affinity was not found in Cos cells that had been engineered to overexpress PC1 (244). This disparity in results is unclear and may depend on differences in the expression levels for PC1 or proteins that bind PC1, including PC2. The required integrity of caveolae for ouabain-mediated effects in ADPKD cells supports the notion that only a subpopulation of caveolae-associated Na-K-ATPase is involved in signaling (115). It is possible that this Na-K-ATPase fraction in ADPKD cells is more sensitive to ouabain. At present, whether the affinity of the Na-K-ATPase for ouabain is influenced by the localization of the enzyme in caveolae or plasmalemma remains unclear. A subpopulation of Na-K-ATPase with high affinity for ouabain was reported in caveolae isolated from circulating levels of ouabain (154). The unique ouabain sensitivity found in ADPKD cells could depend on the aberrant expression of Na-K-ATPase isoforms. However, we determined that this is not the case, since only the α1β1-isozyme could be detected in both ADPKD and NHK cells (154). Alternatively, ouabain affinity may depend on an interaction between Na-K-ATPase and other proteins. The large intracellular region of the Na-K-ATPase located between transmembrane domains 4 and 5 has been shown to interact with the C terminus of polycystin 1 (159, 244). Interestingly, we found that, in insect cells exogenous expression of a construct containing the transmembrane and C-terminal domains of polycystin 1 increased the sensitivity of the Na-K-ATPase to ouabain, with an inhibition constant that was similar to that found in ADPKD cells (21). However, the same change in ouabain affinity was not found in Cos cells that had been engineered to overexpress PC1 (244). This disparity in results is unclear and may depend on differences in the expression levels for PC1 or proteins that bind PC1, including PC2. The required integrity of caveolae for ouabain-mediated effects in ADPKD cells supports the notion that only a subpopulation of caveolae-associated Na-K-ATPase is involved in signaling (115). It is possible that this Na-K-ATPase fraction in ADPKD cells is more sensitive to ouabain. At present, whether the affinity of the Na-K-ATPase for ouabain is influenced by the localization of the enzyme in caveolae or plasmalemma remains unclear. A subpopulation of Na-K-ATPase with high affinity for ouabain was reported in caveolae isolated from normal rat kidneys (61). By contrast, the kinetics for ouabain inhibition of Na-K-ATPase activity was similar between
caveolar and noncaveolar membrane preparations from normal pig kidneys (123). The dissimilarities in results cannot be attributed to different expression of Na-K-ATPase isoforms, since both reports showed the presence of the α9 isoform. However, it is possible that differences in species, or the protocols used for caveolar extraction, may account for the disparity in results. Studies in human ADPKD cells are needed to determine whether specific association of the Na-K-ATPase with components of the caveolae is responsible for the high sensitivity to ouabain that the enzyme has in these cells. If the difference in the ouabain effect between ADPKD cells and normal renal cells is due to differences in ouabain affinity, then it might be expected that higher concentrations of ouabain would have the same effect on NHK cell proliferation and ion transport. However, this does not appear to be the case. It is possible that the concentrations of ouabain required to activate these pathways in normal human renal cells also inhibit pump activity, masking the non-pumping effects of the Na-K-ATPase.

In summary, ouabain accelerates two key pathways in cystogenesis: cell proliferation and Cl−-dependent fluid secretion, through classic intermediates of the Na-K-ATPase signalosome, including EGFR, Src, and the B-Raf/MEK/ERK signaling pathways. This pathway appears to be a common pathway for ADPKD cyst growth, which can also be activated by arginine vasopressin, prostaglandins, and other cAMP agonists (152, 194), epidermal growth factor (EGF) (225), and Src activators (198). We propose that ADPKD cells express a pool of the Na-K-ATPase with an abnormally high affinity for ouabain that causes the cells to respond to endogenous ouabain levels. It is also possible that circulating ouabain levels are elevated in ADPKD patients, which will exacerbate the initial phase of cyst formation and/or promote the continuous growth of the cysts. Interestingly, endogenous cardiotonic steroids have been reported to be elevated in ESRD caused by a variety of kidney pathologies, including PKD (98). Undoubtedly, determination of the endogenous levels of ouabain in patients with ADPKD may shed light on this possibility.

Potential Therapies for ADPKD

Our observations suggest that both endogenous ouabain, as well as ouabain ingested in the diet, may be an important factor in accelerating cyst growth by increasing epithelial cell proliferation and fluid secretion, two key processes in ADPKD pathophysiology. Until a cure to halt cyst formation is available, identification of factors that favor cyst progression provides opportunities to control the advancement and morbidity of the disease. The progress made in understanding the pathogenesis of ADPKD has provided important information on the mechanisms involved in cystogenesis that can be targeted. In particular, efforts have been directed toward interfering with the intracellular pathways governing cyst growth. A more comprehensive review of this topic can be found elsewhere (38, 139, 160, 163, 190, 206, 209, 215). Among the approaches to slowing cyst growth is the vasopressin V2 receptor antagonist tolvaptan, a drug that blocks the renal effect of AVP on cAMP production. Along the same line, increased water intake to suppress AVP levels has been shown to decrease cystic disease in PKD rats (148), and water has been suggested as a potential therapy for ADPKD (208). Somatostatin analogs, such as octreotide, through inhibition of the activity of adenyl cyclase and reduction of intracellular cAMP levels, have also been shown to slow total kidney volume increase in
ADPKD (82). Inhibition of the mTOR pathway, which is abnormally stimulated in ADPKD, with sirolimus has shown beneficial effects in animal models of ADPKD (189); however, it remains unclear whether this is a therapy for patients (184, 188, 217). Targeting of other signaling pathways has also been explored in different animal models of PKD, including EGFR and Src inhibitors EKI-785 and bosutinib (197, 198), and the B-Raf and MEK inhibitors sorafenib and PD184352 (158, 238). The restoration of the abnormal cytosolic Ca\(^{2+}\) levels in ADPKD with calcimimetics or tripiltrope has shown promising results in mice with PKD (108). A different approach consisting of therapies directed to reduce Cl\(^{-}\)-driven fluid secretion with small-molecule CFTR inhibitors, AMPK activation with metformin, or KCa3.1 potassium channel blockers has been explored (7, 109, 134, 241). Finally, other approaches have been directed to stop the hyperproliferative phenotype of the cystic cells using chemotherapeutic agents, such as paclitaxel (131), taxol (226), or cyclin-dependent kinases (34). Due to the complex pathophysiology of ADPKD, it is likely that best treatment will require a combination of therapies. Based on the cystogenic effects of ouabain in ADPKD, it is relevant to propose the development of small-molecule inhibitors to prevent ouabain binding to Na-K-ATPase on cystic cells (59, 112), or inhibiting intracellular pathways activated by ouabain to reduce the progression of the disease. The advantage of targeting the ouabain-Na-K-ATPase signaling pathway is that both cell proliferation and fluid secretion will be affected.

Concluding Remarks

In classic renal physiology, the Na-K-ATPase holds an exalted position as a primary active transporter that is required to translocate Na\(^{+}\) and K\(^{+}\) across the renal tubular epithelium. As if being responsible for life was not enough, more recent evidence indicates that the Na-K-ATPase is also a cell surface receptor for both endogenous and exogenous ouabain and other cardiotonic steroids. In this manner, the Na-K-ATPase is a multifaceted protein involved in regulating several physiological events, once again illustrating that nature is conservative and builds elaborate networks around stable underlying core mechanisms. Understanding the many roles of the Na-K-ATPase is essential to delineating the function of normal kidney physiology and renal pathophysiological states. Studies in ADPKD have given insight into the ion transport and non-pumping functions of the Na-K-ATPase and have revealed a potentially important role for endogenous ouabain in cyst growth. Further investigation of the molecular mechanisms involved in ouabain-Na-K-ATPase signaling on cell proliferation, and cAMP-dependent Cl\(^{-}\) and fluid secretion in ADPKD is still required. The synthesis of novel compounds with the capacity to modulate Na-K-ATPase signaling is in progress, and this may provide new therapeutic approaches that will help ameliorate ADPKD and other renal pathological conditions in which there is aberrant Na-K-ATPase-mediated signaling.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

Author contributions: G.B. and D.P.W. interpreted results of experiments; G.B. and D.P.W. prepared figures; G.B. and D.P.W. drafted manuscript; G.B. and D.P.W. approved final version of manuscript.

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