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ION CHANNELS

The Na/K-ATPase/Src complex and cardiotonic steroid-activated protein kinase cascades

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Abstract The Na/K-ATPase was discovered by Skou in 1957. Since then, the efforts of numerous investigators have led to the following conclusions: (a) This enzyme is indeed the molecular machine for the ATP-dependent and -coupled transport of Na^+ and K^+ across the plasma membrane of a living cell in which such a process (sodium pump) is detected. (b) The Na/K-ATPase is also an important signal transducer that not only interacts and regulates protein kinases, but also functions as a scaffold, capable of bringing the affector and effectors together to form functional signalosomes. This minireview discusses the interaction between the Na/K-ATPase and Src to illustrate how a P-type ATPase can act as a receptor, converting a ligand-binding signal to the activation of protein kinase cascades and the generation of second messengers.

Keywords Na/K-ATPase · Tyrosine kinase · Protein phosphorylation · Receptor · Protein kinase

Abbreviations

A domain	activation domain
ADPKD	autosomal dominant polycystic kidney disease
CD2	second cytosolic domain
CD3	third cytosolic domain
CTS	cardiotonic steroids
EGFR	epidermal growth factor receptor
ERK	extracellular signal-regulated protein kinase
FAK	focal adhesion kinase
FRET	fluorescence resonance energy transfer
GST	glutathione-*S*-transferase
GPCR	G protein-coupled receptor
IP3	inositol triphosphate
IP3R	IP3 receptor
IRBIT	IP_3R binding protein released with inositol 1,4,5-trisphosphate
MBG	marinobufagenin
N domain	nucleotide binding domain
P domain	phosphorylation domain
PI3K	phosphatidylinositol 3-kinase
PKC	protein kinase C
PLC	phospholipase C
PTKs	protein tyrosine kinases
ROMK	renal outer medullary K channel
SERCA	sarcoplasmic reticulum Ca-ATPase

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Introduction

The P-type ATPases control ion fluxes across the cell membrane, whereas membrane receptors convert extracellular signals into the activation of protein kinase cascades as well as the generation of second messengers. When compared, these two groups of functionally divergent membrane proteins do share several common properties. First, many P-type ATPases, like the membrane receptors, interact with membrane and soluble proteins to form large protein complexes in a cell-specific manner. Second, they undergo ligand (substrate) binding-dependent conformational changes. These changes in protein conformation

allow the movement of ions across the cell membrane. They could also alter the function of an interacting protein, and then generate an ion movement-independent cellular action. Consistently, recent studies have demonstrated that the Na/K-ATPase interacts with protein kinases, phosphatase, membrane transporters, and other cellular proteins and that these interactions make the Na/K-ATPase an important signal transducer. For instance, the interaction between the Na/K-ATPase and Src forms a receptor that can confer ligand-like effects of ouabain on protein tyrosine phosphorylation and subsequently on cell growth. This article will first review the background and several key findings that are relevant to the identification of this new cellular signaling mechanism, and then speculate on the potential molecular mechanisms that govern the formation of the Na/K-ATPase/Src receptor complex.

The Na/K-ATPase

The Na/K-ATPase was discovered by Skou as an energy-transducing ion pump that converts the free energy of ATP into transmembrane ion gradients [95]. It belongs to a family of integral membrane proteins called P-type ATPases. The formation of a transient phosphorylated aspartate residue during the catalytic cycle is a hallmark of all P-type ATPase family members. In addition, they undergo a cation-dependent E1 to E2 conformational change that is fueled by ATP hydrolysis and coupled to the ion movement across the cell membrane (for detailed structure, function, and regulation of these ATPases, see reviews [48, 59, 71, 97]). The Na/K-ATPase consists of two non-covalently linked α and β subunits [10, 48, 97]. FXYD proteins, a group of seven structurally similar polypeptides, are expressed in a tissue-specific manner and appear to act as a third subunit of the enzyme. The α subunit (about 112 kDa) contains the ATP and other ligand-binding sites, and is considered as the "catalytic subunit". The β subunit is essential for the assembly of a fully functional enzyme. Several α and β subunits have been identified and functionally characterized [10, 48, 97]. The isoforms are expressed in a tissue-specific manner. The α1 isoform is found in all cells. The α2 and α3 isoforms are expressed in skeletal muscle, neuronal tissue, and cardiac myocytes. The α4 isoform is expressed in the testis and regulates sperm motility [10, 89]. Structural similarities have been found in the non-heavy metal subgroup of P-type ATPases that includes Na/K-ATPase and H/K-ATPase (group IIc) as well as sarcoplasmic–endoplasmic reticulum Ca-ATPase (SERCA, group IIa) [40, 44, 59, 71, 98]. The crystal structures of the SERCA1a at both E1 and E2 states have been resolved [38, 102, 103]. More recently, the structure of the Na/K-ATPase has also been revealed at the E2 conformation [77]. Based on these structures, the Na/K-ATPase α1 subunit, like SERCA1a, contains three distinct functional domains [77, 98]. The A domain consists of the N-terminus and the second cytosolic domain (CD2) connected to transmembrane helices M2 and M3. The α subunit also has the highly conserved phosphorylation (P) domain that is close to the membrane and a relatively isolated nucleotide binding (N) domain. It appears that the A domain rotates while the N domain closes up during the transport cycle, which opens and closes the A, N, and P domains in the E1 and the E2, respectively.

Src, tyrosine phosphorylation and regulation of cell growth by cardiotonic steroids

Cardiotonic steroids A major difference between the Na/K-ATPase and other P-type ATPases is its ability to bind a class of chemicals called cardiotonic steroids (CTS). Although it remains to be firmly established, most biochemical studies have concluded that binding of CTS such as ouabain to the Na/K-ATPase (either phosphorylated or non-phosphorylated) will keep the enzyme at an E2-like close conformation [16, 48, 96]. This action of ouabain is known to inhibit the ion-pumping function of Na/K-ATPase. As discussed in the following section, it also activates the Na/K-ATPase-associated Src, resulting in stimulation of protein tyrosine phosphorylation. Chemically, CTS include plant-derived digitalis drugs such as digoxin and ouabain, and vertebrate-derived aglycones such as bufalin and marinobufagenin (MBG) [1, 91]. While digoxin has been used for more than 200 years to manage congestive heart failure and/or atria fibrillation, bufalin and MBG represent the major active components of a traditional Chinese medicine called Chan'su that is prescribed as a cardiotonic, diuretic, and anodyne agent. Although CTS have been considered only as drugs since their discovery, recent studies have identified both ouabain and MBG as endogenous steroids whose production and secretion are regulated by multiple physiological stimuli including adrenocorticotropic hormone and angiotensin II [28, 36, 57, 61, 91]. Moreover, they are found to play an important role in the regulation of renal salt handling, vascular and cardiac contractions.

In addition to the aforementioned cardiac and vascular effects, CTS play an important role in the regulation of cell growth. In fact, the effects of ouabain on gene expression and the mitogen-induced differentiation and proliferation of lymphoblasts were first noted in the 1970s [24, 47, 85]. Recent studies have greatly expanded the diversity of CTS-affecting cells. Moreover, they showed that CTS activated multiple growth pathways and stimulated either differentiation/apoptosis or hypertrophic/proliferative growth in a cell-type-specific manner [9, 19, 26, 29–35, 41, 49, 56, 60,

62–65, 70, 73, 75, 79, 84, 87, 104]. Significantly, the growth regulatory effects of CTS could occur at nano- and sub-nanomolar concentrations that exhibited no inhibition of cellular Na/K-ATPase pumping activity [6, 53, 60, 64, 86, 90]. Finally, recent studies have demonstrated the potential physiological and pathological significance of CTS-mediated cell growth regulation [29, 50, 81]. To this end, it is important to note the following important studies: First, the effect of sub-nanomolar concentrations of ouabain on cell growth was first observed in cultured canine smooth muscle cells [6]. This observation was subsequently confirmed in several other types of cells including endothelial cells and cardiac fibroblasts [27, 64, 90]. Second, a recent study from Blanco's laboratory suggested that endogenous CTS might play an important role in the pathogenesis of autosomal dominant polycystic kidney disease (ADPKD) [81]. It is known that cyst formation and enlargement require proliferation of mural renal epithelial cells. Blanco and colleagues [81] found that a basal lateral application of sub-nanomolar concentrations of ouabain was sufficient to stimulate the proliferation of ADPKD cells, but not the normal human kidney cells, via the activation of an ERK pathway. Finally, recent in vivo studies have confirmed a role of CTS in the control of cardiac hypertrophy and fibrosis observed in uremic cardiomyopathy [27, 29, 50].

Src and CTS-induced stimulation of protein tyrosine phosphorylation The discovery of a class of proteins (e.g., v-Src and v-Abl) associated with the polyomavirus that phosphorylates tyrosine had revealed the importance of protein tyrosine phosphorylation in the control of cell growth [23]. Accordingly, many studies had been conducted to test whether ouabain and other CTS affected protein tyrosine phosphorylation [6, 22, 34, 55, 56, 58]. These studies demonstrated that sub-toxic concentrations of ouabain indeed stimulated tyrosine phosphorylation of multiple proteins in cardiac myocytes, A7r5 cells, HeLa, and LLC-PK1 cells. These ouabain effects were rapid and dose dependent. Moreover, the addition of a non-specific tyrosine kinase inhibitor such as genistein or herbimycin A was sufficient to block ouabain-induced tyrosine phosphorylation, and subsequently the growth effect of ouabain on cultured cells [6, 34]. More recently, these early observations were confirmed not only in vitro but also in vivo [29, 50]. It is interesting to note that studies have further revealed that increases in protein tyrosine phosphorylation are also important for CTS-induced cellular actions other than cell growth regulation [58, 100, 106, 113].

In principle, increases in protein tyrosine phosphorylation can occur through stimulation of protein tyrosine kinases or inhibition of tyrosine phosphatases or both. The Na/K-ATPase has no intrinsic tyrosine kinase activity. Thus, it is unlikely that the Na/K-ATPase can serve as a receptor tyrosine kinase to convert the ouabain binding to increases in protein tyrosine phosphorylation. Although the α subunit contains a consensus tyrosine phosphatase motif, preliminary studies have shown that it does not have significant tyrosine phosphatase activity (Tian and Xie, unpublished observation). There are many precedents where ligands stimulate protein tyrosine phosphorylation employing receptors that lack intrinsic kinase activity [20, 42, 74]. The examples of this mode of signal transduction include cytokine receptors [42] and G protein-coupled receptors (GPCRs) [74]. While the formation of "binary" cytokine receptors involves non-covalent association of JAK members with the receptor, the GPCRs employ Src family kinases. Thus, it is plausible that non-receptor tyrosine kinases such as Src could also be responsible for ouabain-induced protein tyrosine phosphorylation. Indeed, our initial studies revealed that ouabain increased translocation of cytosolic Src to a Triton-insoluble fraction and stimulated Src activity in cultured cardiac myocytes, A7r5, and LLC-PK1 cells [34]. The stimulation was apparently because of an increase in tyrosine phosphorylation of Src Y418, but not the dephosphorylation of Y529. Moreover, inhibition of Src by several inhibitors blocked ouabain-induced tyrosine phosphorylation of cellular proteins and abolished many ouabain-activated down-stream signal pathways including the activation of ERK. These findings were further confirmed in SYF mouse fibroblasts where Src family kinases were knocked out [35]. Functionally, activation of Src is not only important for CTS to regulate cell growth, but also other cellular activities [45, 52, 58, 67, 69, 82]. To this end, it is of interest to mention that the activated Src appears to be able to phosphorylate the Na/K-ATPase and then regulate the pump activity [11, 12, 29]. Historically, this mode of regulation had been suggested in the 1980s [80].

Identification of Na/K-ATPase/Src complex as a functional CTS receptor

Structure and regulation of Src family kinases Src family kinases are membrane-associated non-receptor tyrosine kinases. The prototype member of the Src family protein tyrosine kinases was first identified as v-Src, the molecule responsible for the cell-transforming ability of the Rous sarcoma virus [14]. So far, at least nine Src family members have been identified and can be divided into two groups: tyrosine kinases with a broad expression (Src, Fyn, Yes) and those mainly expressed in hematopoietic cells (Fgr, Lyn, Hck, Lck, Blk, Yrc, and Yrk) [99]. Structurally, each Src family kinase contains several distinct functional domains (Fig. 1a): (1) the N-terminal Src homology 4 (or SH4) domain, which is involved in targeting Src and other kinases to the membranes through myristoylation and/or palmitoyla-

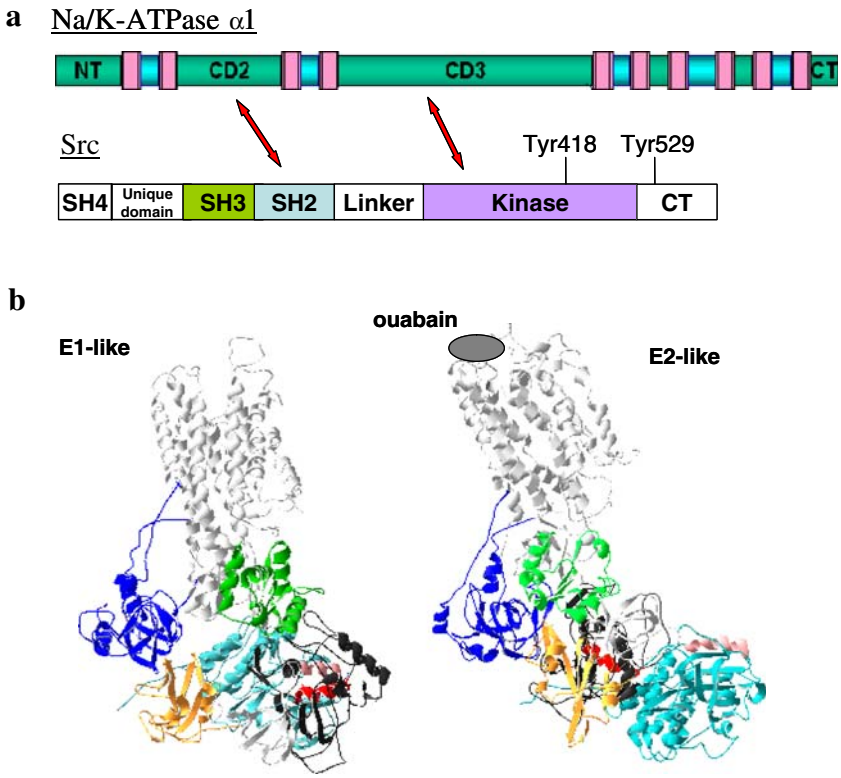

Fig. 1 **a** Schematic presentation of Na/K-ATPase and Src. The identified interacting domains are marked by *arrows*. **b** Modeling of Na/K-ATPase/Src interaction. Modeling of Na/K-ATPase E1- and E2-like structures was based on SERCA1a structure files 1SU4 and 1IWO as well as the newly published Na/K-ATPase structure, and then generated using SPDBView V3.7 program. The A domain (N-terminus and CD2) in Na/K-ATPase was labeled in *blue*, P domain in *green*, N domain in *black*. The SH2 domain of Src was labeled in *orange*, kinase domain in *light blue*. As indicated, we speculate that conformational change from the E1 to the E2 may release the kinase domain, thus activating Src

tion; (2) the SH3, which contains about 60 amino acids and binds the polyproline motifs; (3) the SH2 domain, which interacts with the phosphorylated tyrosine residues; (4) the SH2–Kinase linker region, which can form intramolecular interaction with SH3 and regulate kinase activity; (5) the conserved kinase domain (SH1); and (6) a short C-terminal regulatory tail.

The crystal structures of inactive Src and Hck as well as active Lck have been resolved, and together they provide vivid snapshots of the domain interactions that regulate Src activity [94, 111, 112]. Overall, the kinase domain of the Src family closely resembles that of protein kinase A [37, 54], which consists of an N-terminal and a larger C-terminal lobe linked by the activation loop. The formation of a salt bridge between E312 and K297 (human c-Src sequence) in the N lobe of the kinase domain is required for kinase activity. In addition, kinase activity is regulated by two important and highly conserved tyrosine residues (Y529 and Y418). Phosphorylation of Y529 leads to an intramolecular interaction between the SH2 domain and pY529, which facilitates binding of the SH3 domain to the linker region polyproline type II helix. This SH3-mediated interaction, in turn, inhibits the formation of the salt bridge between E312 and K297, and thus keeps Src in an inactive state. In accordance with the above model, Src can be activated when the interaction between the SH2 domain and pY529 is disrupted because of competitive binding of the SH2 domain to the phosphotyrosine in other proteins such as EGFR. On the other hand, autophosphorylation of Y418 stabilizes the Src in an active state and then stimulates Src activity. Src is known to interact with a number of cellular proteins via various protein domains. The complexities of these interactions suggest that many cellular mechanisms exist for inhibiting/activating the kinase. Examples of Src activators include proteins interacting with the SH2 domain (e.g., platelet-derived growth factor receptor [3] and FAK [21]), SH3 domain (e.g., Nef [76, 105] and Sin [2]), and both SH2 and SH3 (e.g., $p130^{CAS}$ [15]).

The interaction between the Na/K-ATPase and Src The following evidence supports the notion that the Na/K-ATPase directly interacts with Src to form a functional receptor complex in live cells [58, 65, 68, 101, 107]: First, several laboratories, including ours, observed that the Na/K-ATPase and Src were co-enriched in caveolar fractions in different types of cells. Second, immunofluorescence imaging analysis showed co-localization of these two proteins in the plasma membrane. Third, both proteins could be co-immunoprecipitated by either anti-α1 or anti-Src antibodies. Fourth, fluorescence resonance energy transfer (FRET) analysis indicated that both proteins were in close proximity, providing further support of a direct interaction in live cells. Finally, in vitro GST pull-down assay demonstrated direct interactions between the α1

subunit of Na/K-ATPase and Src. As depicted in Fig. 1a, the interaction between the α1 and Src involves at least two contacting sites: one being the CD2 of α1 and Src SH2, and the other consisting of the third cytosolic domain (CD3) of α1 and the Src kinase domain. Based on the predicted structure of α1, the CD2 and CD3 constitute the A and N domains, respectively, and they are highly exposed in the E1 conformation (Fig. 1b). It is interesting to note that both domains are known to interact with structure, membrane, and soluble proteins. Examples of these interactions include ankyrin, inositol 1,4,5-trisphosphate (IP3) receptors (IP3Rs), phosphoinositide 3′ kinase (PI3K), phospholipase C-γ (PLC-γ), and cofilin [7, 43, 62, 113–116].

Functionally, the interaction between the Na/K-ATPase and Src keeps Src in an inactive state. When the molecular mechanism of this inhibition was probed, we found that the CD3/kinase domain interaction was responsible for this inhibition [101]. This is reminiscent of Wiscott–Aldrich syndrome protein-induced Src inhibition [92], but in contrast to the Src kinase domain interaction with Gαs and Gαi, which results in Src activation [72]. It is interesting to note that even though the RACK1/SH2 interaction inactivates Src [17, 18], the CD2/SH2 interaction appears to have no effect on the kinase activity. When the effect of ouabain on these domain interactions was examined, we observed that ouabain reduced the binding of the Src kinase domain, but not the SH2 or full-length Src, to the Na/K-ATPase. Thus, as depicted in Fig. 1b, we suggest that binding of ouabain to the Na/K-ATPase/Src receptor complex may free the Src kinase domain, resulting in activation of the Na/K-ATPase-associated Src kinase. While the Na/K-ATPase provides the ligand (CTS) binding site, the Na/K-ATPase-associated Src functions as a signal transducer, converting as well as amplifying the ligand-binding signal to increases in protein tyrosine phosphorylation. This notion is further supported by the following observations: First, ouabain reduced the FRET efficiency between the Na/K-ATPase and Src in live cells. Concomitantly, it stimulated cellular Src activity. Second, ouabain stimulated the tyrosine phosphorylation of multiple proteins that were either associated with or in close proximity to the Na/K-ATPase/Src receptor complex in a Src-dependent manner. It is of interest to point out that this mode of Src activation is unique and different from other known mechanisms of Src activation induced by either receptor tyrosine kinases or GPCRs. Specifically, it does not require dephosphorylation of pY529 or binding of the Src SH2 to a phosphorylated tyrosine [72, 76, 99]. Moreover, the interaction between the Na/K-ATPase and Src represents a novel regulation of cellular Src activity because of the involvement of both the SH2 and the kinase domains.

To seek additional evidence to support the notion that the Na/K-ATPase/Src complex serves as a CTS receptor, we determined whether a graded-knockdown of cellular Na/K-ATPase could release the Na/K-ATPase-interacting Src, resulting in an increase in basal Src activity and a concomitant loss of ouabain-activated protein tyrosine phosphorylation [65]. Graded knockdown of cellular Na/K-ATPase was achieved by transfecting LLC-PK1 cells with a vector carrying α1-specific siRNA. When basal Src activity was measured, we found that knockdown of the Na/K-ATPase resulted in a significant increase in basal Src activity in a α1 amount-dependent manner. Moreover, tyrosine phosphorylation of FAK, a Src effector, was also elevated. As expected, ouabain failed to stimulate Src and ERK1/2 in the knockdown cells. Furthermore, when the knockdown cells were rescued by rat α1, it not only restored basal Src/FAK activity, but also enabled ouabain to stimulate Src and ERK1/2 at a much higher concentration, consistent with the established differences in ouabain sensitivity between pig and rat α1. Finally, changes in cellular Src/FAK activity in the knockdown cells could also be rescued by expression of a pumping-null rat α1 mutant. Both FRET analysis and co-immunoprecipitation assay showed that the pumping-null mutant was fully capable of interacting with Src. It is important to note that this mode of interaction appears to operate in vivo as well since Src activity in tissues harvested from α1+/− mice were significantly increased (Li and Xie, unpublished data). Taken together, the data indicate that there is a pool of the Na/K-ATPase-interacting Src in live cells. The interaction between the Na/K-ATPase and Src not only regulates basal Src activity, but also serves as a function receptor for CTS to stimulate Src and subsequently protein tyrosine phosphorylation.

Despite progress in understanding the Na/K-ATPase/Src receptor complex, several important issues are worthy of further discussion. First, the Src family consists of at least nine members and many of them are expressed in a tissue-specific manner. Since these kinases are highly conserved, we suggest that the Na/K-ATPase may interact with other members of Src family kinases. We further speculate that these interactions may constitute the tissue-specific receptor Na/K-ATPase/Src family kinase complex that provides a certain degree of signaling specificity. Second, there are at least four α isoforms. Because these isoforms are highly conserved, it is most likely that α isoforms other than α1 would interact with Src or Src family kinases to form a receptor complex. To this end, it is of interest to mention that Src also interacts with H/K-ATPase [101], potentially forming yet another group of receptor complexes. Moreover, recent studies have demonstrated that both Na/K-ATPase and H/K-ATPase are effectors of Src family kinases [46]. All together, the diversity of these interactions could produce a large population of receptor species and provide a tissue-specific response. Needless to say, these issues have to be further investigated.

Signal amplification, conversion, and termination Because CTS bind and consequently inhibit the pumping function of the Na/K-ATPase, they have been considered as specific pump inhibitors. Accordingly, the primary effects of CTS on various cellular functions have been ascribed to the inhibition of the Na/K-ATPase, and subsequent changes in intracellular ion concentration. In contrast, CTS would activate the Na/K-ATPase/Src receptor complex, thus making CTS true agonists of the receptor. Functionally, activation of this receptor complex by CTS results in signal amplification and conversion via the stimulation of protein kinase cascades and generation of second messengers. For example, it has been reported that binding of ouabain to this receptor complex was capable of recruiting and transactivating the EGFR [6, 34, 35, 58, 60]. This apparently involved Src-dependent phosphorylation of the EGFR at sites other than the major autophosphorylation site Y1173 [35]. The trans-activated EGFR, in turn, recruited adaptor protein Shc to the complex, resulting in activation of the Ras/Raf/MEK/ERK cascade [6, 35]. Similarly, Src activation also led to stimulation of PLC-γ and subsequent activation of protein kinase C (PKC) isozymes and IP3-mediated Ca^{2+} signaling [113]. Moreover, ouabain was found to stimulate PI3K, which works in concert with PKC, resulting in endocytosis of this receptor complex. This explains how cells terminate the ouabain-activated signal transduction [67]. In short, the identified Na/K-ATPase/Src complex appears to function as other receptor tyrosine kinases, capable of amplifying and converting signals as well as terminating the CTS-provoked signal transduction. Clearly, the identification of this new cellular signaling mechanism has provided new insight into the molecular action of CTS. It has also established a new target for developing novel agonists and antagonists of this receptor complex. To this end, recent studies have shown that some derivatives of ouabain such as rostafuroxin indeed antagonize the effect of ouabain on Src, but have no effect on Na/K-ATPase activity [29].

Although many laboratories have documented that changes in protein interaction are sufficient for CTS to provoke signal transduction, it is important to point out that these new findings do not exclude a role of the ion-pumping function of Na/K-ATPase in CTS-induced effects. Because the Na/K-ATPase is functionally coupled to many ion transporters such as the Na^+/Ca^{2+} exchanger, localized inhibition of the pumping function will modulate function of the Na^+/Ca^{2+} exchanger and increases intracellular Ca^{2+}, especially in cells such as cardiac myocytes where the Na^+/Ca^{2+} exchanger is highly expressed. Thus, we believe that both processes could work in concert in the regulation of cellular function such as myocardial contractility. Indeed, recent studies have demonstrated that either knockout of Na^+/Ca^{2+} in cardiac myocytes or inhibition of the Src/PLCε pathway is effective in blocking ouabain-induced inotropy [4, 51, 82, 88]. Moreover, since intracellular Na^+ regulates the conformation of the Na/K-ATPase (e.g., the E1 state), it is plausible that changes in intracellular Na^+ concentration could also regulate the formation of the Na/K-ATPase/Src complex, thus cellular Src activity. Clearly, these issues remain to be experimentally resolved.

It is important to note that many ion transporters and channels, like the Na/K-ATPase, are capable of forming signaling complexes and then serving as receptors or as signal integrators. Examples for such roles of ion-transporting proteins include, but are not limited to, the Na^+/H^+ exchanger 1 [8], transient receptor potential channels [25, 108], the red blood cell anion exchanger band 3 [13], and IP3Rs [5, 83, 93]. For instance, IP3R interacts and keeps a protein called IP3R binding protein released with IP3 (IRBIT) in an inactive state. Binding of IP3 to the IP3R changes the conformation of the receptor, resulting in release of IRBIT from IP3R, which subsequently binds and stimulates the $Na^+ / vlldelimiterspace HCO_3^-$ co-transporter in renal epithelial cells [5, 93].

Molecular mechanism of receptor formation As depicted in Fig. 1b, modeling of interactions between the Na/K-ATPase and Src has led us to propose the following working hypotheses: First, we believe that both CD2/SH2 and N domain/kinase domain interactions could simultaneously occur because our in vitro studies showed that ouabain did not reduce binding of Src to the Na/K-ATPase [101]. Second, based on the crystal structures of Src, Na/K-ATPase, and SERCA, we believe that formation of the Na/K-ATPase/Src receptor complex would occur (or stabilize the Na/K-ATPase) in an E1-like conformation. We further suggest that the Src kinase domain would dissociate from the Na/K-ATPase in an E2-like conformation because of rotation of the A domain and the inward movement of the N domain (Fig. 1b). Specifically, we believe that this is how ouabain activates the Na/K-ATPase-associated Src. Third, we found that the CD2/SH2 interaction had a higher affinity than that of the N domain/kinase domain interaction (Li and Xie, unpublished data). Therefore, we contend that the CD2/SH2 interaction would facilitate the interaction between the N domain and the kinase domain, thus helping the formation of the Na/K-ATPase/Src receptor complex. Moreover, when ouabain turned the Na/K-ATPase into an E2-like conformation [16, 96], rotation of the A domain (i.e., CD2) would work in concert with the movement of the N domain, resulting in the release of the Src kinase domain and thus the activation of Src. Therefore, we propose that formation of a functional receptor Na/K-ATPase/Src complex requires both CD2/SH2 and N domain/kinase domain interactions. Needless to say, this proposal has to be experimentally tested.

Conclusions and perspectives

Studies from many laboratories of the past few years have documented that Na/K-ATPase has a receptor function that confers a ligand-like effect of CTS on protein tyrosine phosphorylation in addition to the well-described ion-pumping function. Meanwhile, there is also increased appreciation of other membrane transporters in signal transduction. These studies certainly mark the beginning of a fascinating new field of investigation, evidenced by rapid growth of research concerning the role of these membrane proteins in signal transduction. Many important issues, especially those related to mechanism and physiological significance, remain to be resolved. For instance, does E1 and E2 conformational cycling regulate the interaction between the Na/K-ATPase and Src as predicted in Fig. 1b? Resolving this issue will certainly enhance our understanding of the molecular mechanism of CTS action. It may also reveal an intimate relationship between ion pumping and signal transduction. In principle, the Na/K-ATPase/Src complex could continuously generate signals through E1 and E2 cycling when it pumps. This mechanism, if proven to be true, would connect two basic cellular activities (pumping and signal transduction) together, which may be relevant to a well-documented cellular signaling mechanism of "pump–leak" coupling [39, 78, 109, 110]. It is known that apical and basolateral transport activities are tightly balanced, and changes in basolateral Na/K-ATPase pumping activity directly affect the apical cation conductance in renal epithelial cells. It was recently reported that activation of Src led to decreased exocytosis of renal outer medullary K channels (ROMK) to the apical membrane [66]. Thus, changes in the pumping activity (E1/E2 cycling) could affect Src activity, resulting in a feedback regulation of cellular leaks (e.g., ROMK-mediated K^+ efflux).

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