Na⁺-, ouabain-, Ca²⁺-, and thapsigargin-sensitive ATPase activity expressed in chimeras between the calcium and the sodium pump α subunits

(Na⁺,K⁺-ATPase/Ca²⁺-ATPase/cardiac glycoside/thapsigargin/chimeric molecule)

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ABSTRACT Using the chicken sarcoplasmic/endoplasmic reticulum Ca²⁺ (SERCA)-ATPase as a parental molecule and replacing various portions with the corresponding portions of the chicken Na⁺,K⁺-ATPase α_1 subunit, Ca²⁺/thapsigargin- and Na⁺/ouabain-sensitive domains critical for these P-type ATPase activities were identified. In the chimera, [n/c]CC, the amino-terminal amino acids Met-1 to Asp-162 of the SERCA (isoform 1) (SERCA1) ATPase were replaced with the corresponding portion (Met-1–Asp-200) of the Na⁺,K⁺-ATPase α_1 subunit. In the chimera CC[c/n], the carboxyl-terminal amino acids (Ser-830 to COOH) of the SERCA1 ATPase were replaced with the corresponding segment (Leu-861 to COOH) of the Na⁺,K⁺-ATPase α_1 subunit, and in the chimera CNC, the middle part (Gly-354–Lys-712) of the SERCA1 ATPase was exchanged with the Na⁺,K⁺-ATPase α_1 subunit (Gly-378–Lys-724). None of the chimeric molecules exhibited any detectable ouabain-sensitive Na⁺,K⁺-ATPase activity, but they did exhibit thapsigargin-sensitive Ca²⁺-ATPase activity. Therefore, the segments Ile-163–Gly-354 and Lys-712–Ser-830 of the SERCA1 ATPase are sufficient for Ca²⁺ and thapsigargin sensitivity. The SERCA1-ATPase activity of [n/c]CC, but not of CCC, CNC, or CC[c/n], was further stimulated by addition of Na⁺ in the assay medium containing Ca²⁺. This additional stimulation of SERCA1-ATPase activity by Na⁺ was abolished when the amino-terminal region (Met-1–Leu-69) of [n/c]CC was deleted ([Δ n/c]CC). In the absence of Na⁺, the SERCA1-ATPase activity of [n/c]CC was inhibited by ouabain, and, in the presence of Na⁺, its activity was stimulated by this drug. On the other hand, the ATPase activity of [Δ n/c]CC was not affected by ouabain, although [Δ n/c]CC can still bind [³H]ouabain. These results suggest that a distinct Na⁺-sensitive domain (Na⁺ sensor) located within the restricted amino-terminal region (Met-1–Leu-69) of the Na⁺,K⁺-ATPase α_1 subunit regulates ATPase activity. The Na⁺ sensor also controls ouabain action in concert with the major ouabain-binding region between Ala-70 and Asp-200 of α_1 subunit.

The plasma membrane Na⁺,K⁺-ATPase and the sarcoplasmic/endoplasmic reticulum Ca²⁺-(SERCA) ATPase belong to a family of P-type ATPases that undergo a cycle of conformational changes between phosphorylated and dephosphorylated stages (1, 2). Major research effort for the past years has been directed toward identification of specific ion- and inhibitor-binding domains (3). Point mutations and chemical modification in transmembrane segments have provided initial evidence for functional domains responsible for inhibitor (4–9) and ion binding (10–12) by these ATPases. More recently, the use of chimeric ATPases with characteristics of distinct ion pumps for functional studies has become possible (13–18). Taking advantage of the limited conserva-

tion of their amino acid sequences (14) and the similar distribution of hydrophobic domains throughout the molecules (15), a series of chimeric ATPases were constructed (15–17). Functional comparison of these chimeric molecules should provide critical information about the structure–function relationships of the SERCA ATPase and the Na⁺,K⁺-ATPase. In this report, we examined the effects of Na⁺, Ca²⁺, ouabain, and thapsigargin on a series of chimeric and wild-type molecules side by side in transfected mouse L cells.

MATERIALS AND METHODS

Expression of Chicken Chimeric cDNAs in Mouse L Cells. Fig. 1 schematically represents the wild-type and chimeric ATPases expressed in this study. The wild-type and chimeric chicken cDNA constructs encoding NNN (19), CCC (20), [n/c]CC (16), CNC (17), CC[c/n] (15), and [Δ n/c]CC (C and c stand for parts of SERCA1 ATPase, N and n stand for parts of Na⁺,K⁺-ATPase, and Δ n represents a deleted portion of n) were cloned into mammalian expression vector pRc/CMV (Invitrogen, no. V750-20), introduced into mouse Ltk⁺ β cells that had been transfected with a cDNA encoding the chicken Na⁺,K⁺-ATPase β_1 subunit (21), and G418-resistant colonies were isolated. At least 10 different cell lines per cDNA construct were selected on the basis of high levels of immunofluorescence staining (15, 21) and subjected to immunoprecipitation to identify individual cell lines that expressed chicken chimeric molecules at a similar level. In immunological detection, monoclonal antibody (mAb) IgG 5 specific to chicken Na⁺,K⁺-ATPase α subunit (19) was used for NNN and CNC, and mAb IgG 5D2 specific to chicken SERCA1 ATPase (22) was used for CCC, [n/c]CC, [Δ n/c]CC, and CC[c/n]. The encoding cDNA for [Δ n/c]CC was constructed by replacing the first 207 bp encoding Met-1–Leu-69 of the Na⁺,K⁺-ATPase portion in the [n/c]CC chimera with an artificial start codon, ATG. For this replacement, the fragment between the artificial start codon and the unique recognition site for endonuclease *EcoRV* at the chimeric junction was amplified by oligonucleotide-directed mutagenesis using PCR and inserted into the *EcoRV* site in [n/c]CC. For PCR, 50 ng each of the synthetic primers 5'-GCACCATGGCTCGTGATGGCCCAAATCCCT-3' (artificial start codon is underlined) and 5'-CCGGATATCAGCTGGAATTCT-3' (*EcoRV* site is underlined) and 50 ng of the chicken Na⁺,K⁺-ATPase α_1 subunit cDNA were used, and

Abbreviations: SERCA, sarcoplasmic/endoplasmic reticulum Ca²⁺; SERCA1, sarcoplasmic/endoplasmic reticulum Ca²⁺-ATPase (isoform 1); mAb, monoclonal antibody.

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FIG. 1. Schematic representation of wild-type and chimeric proteins. (Upper) Working model for structure and function of the Na⁺,K⁺-ATPase α subunit that accommodates all results with SERCA ATPase as a parental molecule. The model includes Na⁺ sensor, ouabain-binding, and subunit-assembly domains. The topology model proposed for the wild-type sarcoplasmic reticulum Ca²⁺-ATPase (10) is adapted to all constructs on the basis of reported results (13–17). (Lower) Wild-type Na⁺,K⁺-ATPase (NNN), [n/c]CC, [Δn/c]CC, CNC, CC[c/n], and wild-type Ca²⁺-ATPase (CCC) are shown with the total amino acid numbers of 1021, 1032, 964, 983, 991, and 994, respectively. Chimeric junctions (○) are shown by three-letter notation: asp200/ILE163 for [n/c]CC, GLY354/thr378 and LYS712(lys724) for CNC, and SER830/leu861 for CC[c/n]—where asp200, thr378, lys724, and leu861 come from NNN; and ILE163, GLY354, LYS712, and SER830 originate in CCC. The numbers of amino acids between these chimeric junctions are shown in NNN and CCC.

nucleotide sequences obtained were confirmed by DNA sequencing (23).

Metabolic Labeling and Immunoprecipitation. Immuno-beads coupled with mAbs (IgG 5D2 and IgG 5) were prepared by reductive amination reaction as described (15, 24). Cells (5×10^5 cells per 60-mm culture dish) were labeled in methionine-free Dulbecco's modified Eagle's medium (GIBCO) containing [³⁵S]methionine/[³⁵S]cysteine (Tran³⁵S-label, ICN) at 20–40 μCi/ml (1 Ci = 37 GBq) for 12 h and then solubilized in 1% Triton X-100/150 mM NaCl/50 mM Tris-HCl, pH 7.4/5 mM EGTA/2 mM phenylmethylsulfonyl fluoride at 4°C. The solubilized materials (0.6 mg protein per ml) were subjected to affinity chromatography with Immuno-beads as described (15, 19), and the purified materials were analyzed by SDS/7.5% PAGE and fluorography.

ATPase Activities and [³H]Ouabain Binding. The Na⁺,K⁺-ATPase activity was defined as the ouabain- (5 mM) inhibitable ATP hydrolysis in 100 mM NaCl/5 mM KCl/50 mM Tris-HCl, pH 7.4/2 mM ATP (containing 100 μM NaOH and [³²P]ATP/3 mM MgCl₂/5 mM NaN₃/1 mM EGTA (pH 7.4

with ≈260 μM NaOH). The SERCA-ATPase activity was defined as the thapsigargin- (500 nM) inhibitable Ca²⁺-dependent ATP hydrolysis in 100 mM KCl/50 mM Tris-HCl, pH 7.4/2 mM ATP (containing 100 μM KOH and [³²P]ATP), 3 mM MgCl₂, 2 μM ionophore A23187 (Boehringer Mannheim), 1 mM NaN₃, and appropriate amounts of CaCl₂ and EGTA (25). Crude membrane preparations [≈1 mg of protein per ml in TME buffer (75 mM Tris-HCl, pH 7.5/12.5 mM MgCl₂/1.5 mM EDTA)] were obtained from monolayers of cells and used for these ATPase assays (60 ng protein per assay) as described (16). Specific ouabain binding to the crude membrane preparation was measured as described (16).

RESULTS

Expression of Chicken Chimeric Molecules in Mouse L Cells. All chicken cDNA constructs were stably transfected into mouse L cells that had been expressing the chicken β₁ subunit (21), because one of the chimera, CC[c/n], was known to assemble with the β subunit (15). The immunoprecipitation profiles of the wild-type (NNN and CCC) and chimeric molecules ([n/c]CC, [Δn/c]CC, CNC, and CC[c/n]) in six selected cell lines indicated that these chicken molecules are expressed at a similar level (Fig. 2), and, therefore, these cell lines were used for further functional studies.

All Chimeric ATPases Exhibited SERCA-ATPase Activity But No Na⁺,K⁺-ATPase Activity. None of the chimeric molecules ([n/c]CC, CNC, and CC[c/n]) exhibited any detectable ouabain-sensitive Na⁺,K⁺-ATPase activity over the background of endogenous mouse enzyme (data not shown) but did exhibit SERCA-ATPase activity, defined as the thapsigargin-inhibitable Ca²⁺-dependent ATP hydrolysis (27–30), at least 300–500% higher than that of mouse endogenous enzyme at optimum free Ca²⁺ concentrations of 1–5 μM (Fig. 3A). The background level of mouse endogenous SERCA-ATPase activity does not change in NNN- (wild-type Na,K-ATPase) transfected mouse L cells (16). These differences in activities in transfected and control mouse cells can be easily and repeatedly identified due to the advantage

FIG. 2. Immunoprecipitation of chicken proteins in transfected mouse L cells. The ³⁵S-labeled proteins in cloned cells were immunoprecipitated by using monoclonal antibodies, IgG 5 specific for the chicken Na⁺,K⁺-ATPase α subunit (A) and IgG 5D2 specific for the chicken SERCA1 (B), and subjected to SDS/PAGE analysis and then fluorography. Because use of two different monoclonal antibodies was necessary to detect all constructs, the expression levels cannot be directly compared between the two sets of immunoprecipitation. However, there are several lines of evidence that the expression levels of these selected cell lines are essentially equal. (i) [n/c]CC, [Δn/c]CC, CCC, and CC[c/n] are expressed equally, as evident by the similar amount of molecules in cells (A). (ii) NNN and CNC are also expressed equally (B). (iii) The number of [³H]ouabain-binding sites in cells expressing NNN (detected by mAb IgG 5 in B) and [n/c]CC (detected by mAb IgG 5D2 in A) has been found equivalent (16), and these same cell lines are used here. (iv) The numbers of methionine and cysteine residues that can be metabolically labeled in the expressed molecules are similar: 51 in CCC, 50 in NNN, 57 in [n/c]CC, 54 in [Δn/c]CC, 43 in CNC, and 48 in CC[c/n]. Thus, expression levels of all constructs are estimated to be very similar.

FIG. 3. (A) SERCA-ATPase activity expressed in transfected mouse L cells. SERCA-ATPase activities of NNN-transfected cells at various Ca^{2+} concentrations were essentially identical to those of mouse Ltk cells (data not shown). All data points represent mean $\pm$ SD of four to six separate experiments. *, Significance level ($P < 0.01$) compared with mouse endogenous enzyme. (B) Thapsigargin sensitivity of SERCA-ATPase activity in transfected mouse L cells. Typical examples at 5 μM free Ca^{2+} are shown for each cell line.

of our stable expression system, which allows us to express all activity as nmol/min per mg of proteins (not as percentage maximum, except in Fig. 3B).

The inhibitory effect of thapsigargin is dose-dependent, and a single IC_{50} value of ≈ 1 nM for thapsigargin was obtained in all cell lines (Fig. 3B), indicating that the integrity of the site(s) responsible for thapsigargin binding and inhibition was preserved in these chimeras. The IC_{50} value of thapsigargin can vary, depending upon the amount of proteins in assay systems (28); therefore, it is often expressed as nmol/mg of protein (29, 30). In our study, IC_{50} values were determined in the presence of ≈ 60 ng of protein per assay (see *Materials and Methods*), and a common IC_{50} value corresponding to 8 pmol/mg of protein was obtained for all cell lines. These data also verify a similar level of expression of exogenous proteins in transfected cells.

Na^+ Sensitivity of Chimeric ATPases. The SERCA-ATPase activity of [n/c]CC in transfected mouse L cells was significantly stimulated toward the level of wild-type Ca^{2+} -ATPase

(CCC) with increased concentrations of Na^+ ions in the assay medium, whereas the SERCA-ATPase activity of CCC, CNC, CC[c/n], or the endogenous mouse Ca^{2+} -ATPase was not affected by Na^+ ions (Fig. 4). It should be noted that Na^+ cannot activate [n/c]CC without Ca^{2+} because Ca^{2+} is essential for the ATPase activity of [n/c]CC (Fig. 3).

Although the SERCA ATPase and the Na^+, K^+ -ATPase α subunits have similar higher-order structures across the membrane (32, 33), the amino terminus of the Na^+, K^+ -ATPase α subunit is ≈ 50 amino acids longer than that of the SERCA ATPase, and these amino acids are linked to the rest of the molecule via a short proline-rich bridge (-Pro-74-Asn-75-Thr-76-Leu-77-Thr-78-Pro-79-Pro-80-Pro-81-Thr-82-Thr-83-Pro-84-) (19). This amino-terminal region is also characterized

FIG. 4. (A) Amino-terminal 69 amino acids of the Na^+, K^+ -ATPase act as a Na^+ sensor. SERCA-ATPase activity was measured at 5 μM free Ca^{2+} and different Na^+ concentrations. Total ionic strength was kept at 180 mM with 100 mM KCl and 80 mM of NaCl/choline chloride (31). The SERCA-ATPase activity of [n/c]CC was regulated by Na^+ ions, whereas the activity of CCC, CNC, CC[c/n] or mouse endogenous enzyme was unaffected. Removal of the amino-terminal 69 amino acids abolished sensitivity to Na^+ ions. The ED_{50} value for Na^+ ions for stimulation of [n/c]CC (≈ 50 mM) is within the physiological local concentrations of Na^+ after an action potential, although the value is slightly higher than that (10–20 mM) for wild-type Na^+, K^+ -ATPase (31). The SERCA-ATPase activities measured at high-ionic strength with 80 mM choline chloride were $\approx 50\%$ of activities at normal conditions (compare with Fig. 3). All data points represent mean $\pm$ SD of separate experiments ($n = 10$ for [n/c]CC, and $n = 4$ for others). *, Significance level ($P < 0.01$) compared with values at 0 mM Na^+ . (B) Ouabain-binding ability of [Δ n/c]CC was still retained (compare with no ouabain binding in mouse Ltk cells within the same [^3H]ouabain-concentration range), further limiting the location of ouabain-binding sites to the region between Leu-69 and Asp-200 in the Na^+, K^+ -ATPase α_1 subunit. All data points represent mean $\pm$ SD of four separate experiments.

by its hydrophilic nature and the abundance in lysine residues (1, 16, 32). Although there is no direct evidence, this region may be involved in ion binding/transport (34–36). When these amino acids (Met-1–Leu-69) were deleted from [n/c]CC, forming the [Δ n/c]CC chimera, the SERCA-ATPase activity became insensitive to Na⁺ ions (Fig. 4).

Ouabain Sensitivity of Chimeric ATPases. [³H]Ouabain at the range of 1–800 nM did not bind to either the wild-type SERCA1 (CCC) or the chimeras CC[c/n] and CNC but did bind to the wild-type Na⁺,K⁺-ATPase (NNN) and the chimeras ([n/c]CC and [Δ n/c]CC). The [n/c]CC chimera binds [³H]ouabain very similarly to the NNN (16). However, binding of [³H]ouabain to [Δ n/c]CC indicated a rather complex mechanism (Fig. 4B), although it demonstrated a large amount of specific binding.

Without Na⁺, ouabain inhibited the SERCA-ATPase activity of [n/c]CC in a dose-dependent way (Fig. 5A), as reported (16). On the other hand, in the presence of Na⁺ (80 mM), ouabain enhanced the SERCA-ATPase activity of this chimera (Fig. 5B). These positive and negative effects of ouabain with and without Na⁺ were abolished in chimera [Δ n/c]CC (Fig. 5), even though this latter chimera can bind ouabain (Fig. 4).

FIG. 5. Ouabain sensitivity of the SERCA-ATPase activities expressed in chimeric and wild-type molecules. Effects of ouabain were measured with (A) and without (B) 80 mM NaCl in the SERCA-ATPase assay system (16, 25). All data points represent mean $\pm$ SD of separate experiments ($n = 10$ for [n/c]CC, and $n = 4$ for the rest). *, Significance level ($P < 0.01$) compared with values at 0 μ M ouabain. The wild-type SERCA-ATPase activities at higher ionic strength (i.e., with Na⁺) are lower than those without Na⁺ (compare A and B). The activity of [n/c]CC is higher than that of [Δ n/c]CC in B due to the stimulating effect of Na⁺, as seen in Fig. 4A.

DISCUSSION

Our heterologous expression system exploits the high specificity of thapsigargin to SERCA ATPase. Applying Ca²⁺-/Na⁺-pump chimeras to this system, the domains within the SERCA ATPase responsible for activation by Ca²⁺ and inhibition by thapsigargin were identified. The amino-terminal 69 amino acids of the Na⁺,K⁺-ATPase α_1 subunit functioned as a Na⁺ sensor. This Na⁺ sensor can regulate ouabain binding to the region between Ala-70 and Asp-200. How Na⁺ binding to the sensors relates to Na⁺ transport remains to be resolved.

Minimum Requirement for SERCA-ATPase Activity. The CNC possesses ATP-hydrolysis activity (Fig. 3) and can be phosphorylated (17) in a Ca²⁺- and thapsigargin-dependent fashion. This result suggests that the middle ATP-catalytic domain is unlikely to be responsible for the sensitivity to thapsigargin and Ca²⁺; thapsigargin should interact with Met-1–Gly-354 and/or Lys-712–carboxyl end of the SERCA1. Because thapsigargin competes with Ca²⁺ in ATP catalysis (29), it is reasonable to conclude that the membranous amino- and carboxyl-terminal regions of the sarcoplasmic reticulum Ca²⁺-ATPase are critical for SERCA-ATPase activity.

The [n/c]CC and CC[c/n] chimeras are also thapsigargin- and Ca²⁺-sensitive ATPases (Fig. 3). These results further restrict location of the domains responsible for thapsigargin inhibition; the segments between Ile-163 and Gly-354 and/or between Lys-712 and Ser-830 of SERCA1 are sufficient for the thapsigargin- and Ca²⁺-sensitive ATPase activity. These potential regions contain the transmembrane segments M3, M4, M5, and/or M6. Recent studies using point mutations of charged amino acids in these transmembrane domains also suggest the importance of these regions (37, 38). The chimera NCC, where an additional segment containing M3–M4 was replaced with the corresponding region of the Na⁺,K⁺-ATPase α subunit, failed to exhibit Ca²⁺- and thapsigargin-sensitive ATPase activity (16).

The SERCA-ATPase activity in all cell lines tested exhibited a similar Ca²⁺-dependence, although the activities of chimeras were significantly lower than the wild type, CCC. This result indicates that Ca²⁺ actually binds to the chimeric molecules and initiates a series of conformational changes associated with ATP hydrolysis, although with lower efficiency. A recent study on chimeric molecules between Ca²⁺-ATPase isoforms (SERCA/1, -2, and -3) has suggested that the middle segment of the SERCA ATPases is responsible for cooperative interaction between Ca²⁺ binding and ATP hydrolysis (39). Our results suggest that modifications at the amino- or carboxyl-terminal regions may also influence such a long-range conformational change.

The Region Between Met-1 and Leu-69 Is a Sensor for Na⁺. The SERCA-ATPase activity of [n/c]CC was significantly stimulated with increased Na⁺ concentrations in the assay medium, whereas the SERCA-ATPase activity of CCC, CNC, CC[c/n], or the endogenous mouse Ca²⁺-ATPase was unaffected (Fig. 4). These results indicate that the amino-terminal 200 amino acids of the Na⁺,K⁺-ATPase α_1 subunit contain a domain that plays a role as a sensor for Na⁺ ions and confers Na⁺ sensitivity on the SERCA ATPase. This idea is supported by the fact that, when the amino-terminal 69 amino acids (Met-1–Leu-69) were deleted, the Na⁺ sensitivity of the SERCA-ATPase activity was lost (Fig. 4).

The Na⁺,K⁺-ATPase tightly binds K⁺ in the absence of Na⁺ (40). This phenomenon has been termed "K⁺ occlusion" and is thought important for Na⁺ and K⁺ transport. A recent report (33) suggested that a 19-kDa fragment derived from the carboxyl terminus of the Na⁺,K⁺-ATPase is the site for K⁺ occlusion. The chimera CC[c/n], which includes the 19-kDa fragment, did not show Na⁺ sensitivity in the present assay system containing 100 mM K⁺ (Fig. 4), whereas this

chimera exhibited distinct K^+ sensitivity very similar to that of wild-type Na^+, K^+ -ATPase in the absence of Na^+ (preliminary results appear in ref. 41). On the other hand, the chimera [n/c]CC exhibited Na^+ -sensitive SERCA-ATPase activity, although it required free Ca^{2+} for its basic activity. Thus, how the Na^+ binding to the sensor relates to Na^+/K^+ occlusion and transport is now a fascinating question.

Complexity of Ouabain Binding/Inhibition. The [n/c]CC chimera can bind ouabain without the Na^+, K^+ -ATPase β subunit (15, 16). A deletion mutant, [Δ n/c]CC, which also lacked the assembly domain with the β subunit (15), still retained ouabain-binding ability (Fig. 4). However, the SERCA-ATPase activity of [Δ n/c]CC was insensitive to ouabain, whereas the [n/c]CC activity was ouabain-sensitive (Fig. 5). The complexity of ouabain effect on the Na^+, K^+ -ATPase α subunit has been noted (42) in conjunction with the role of the amino-terminal domains (Met-1 to Leu-266) of the canine α subunit. Our present results further define the functional amino-terminal domain and suggest that the region between Ala-70 and Asp-200 of the chicken Na^+, K^+ -ATPase α_1 subunit is sufficient for ouabain binding, but it requires other regions for subtle regulation—e.g., the amino-terminal cytoplasmic region (Met-1–Leu-69) influences the ouabain-binding and -inhibitory behaviors.

The property of ouabain binding can be changed by a variety of point mutations within regions other than the amino-terminal 200 amino acids (7, 9). The ouabain-sensitive function of the Na^+, K^+ -ATPase α subunit is also modulated by assembly with the β subunit (43–45), possibly through conformational changes in remote regions of the α subunit upon subunit assembly. It is noteworthy that the carboxyl-terminal 161 amino acids of the α subunit (which include M7 and M8) are sufficient for assembly with the β subunit (15) and that the ouabain-binding property of the α subunit is affected by a point mutation introduced between M7 and M8 (7). As discussed when noting the importance of the amino- and carboxyl-terminal regions of the SERCA ATPase in activity, the amino- and carboxyl-terminal regions of the Na^+, K^+ -ATPase α subunit probably influence each other in ATPase function, especially in ouabain binding.[‡]

[‡]The intestinal H^+, K^+ -ATPase has been reported ouabain sensitive (46–48). In this regard, a recent report on ouabain-sensitive ^{86}Rb flux in a chimeric molecule consisting of the amino-terminal half of the gastric H^+, K^+ -ATPase α subunit and the carboxyl-terminal half of the Na^+, K^+ -ATPase α subunit (18) is intriguing. Because the H^+, K^+ - and Na^+, K^+ -ATPases exhibit a high degree (>65%) of amino acid identity (49, 50), it is reasonable to speculate that the H^+, K^+ -ATPase, in general, possesses a potential ouabain-binding site(s), which can be converted to a true ouabain site(s) by interaction with proper structural signals from elsewhere in the molecule.

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