Identification of a Region within the Na,K-ATPase α Subunit That Contributes to Differential Ouabain Sensitivity

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To analyze determinants within the Na,K-ATPase α subunit that contribute to differential ouabain sensitivity, we constructed and expressed a panel of chimeric cDNA molecules between ouabain-resistant and ouabain-sensitive α subunit cDNAs. When introduced into ouabain-sensitive monkey CV-1 cells, ouabain-resistant rat $\alpha 1$ subunit cDNA and chimeras in which the 5' end of ouabain-sensitive human $\alpha 1$ or rat $\alpha 2$ subunit cDNA was replaced by the 5' end of rat $\alpha 1$ subunit cDNA conferred resistance to 100 μ M ouabain. Monkey cells transfected with the reciprocal chimeras were unable to survive selection in 1 μ M ouabain. Rat $\alpha 2$ subunit cDNA and a chimera in which the 5' end of rat $\alpha 1$ subunit cDNA was replaced by the 5' end of rat $\alpha 2$ subunit cDNA conferred resistance to 0.5 μ M ouabain. These results suggest that determinants of ouabain resistance reside within the amino-terminal portions of the rat $\alpha 1$ and $\alpha 2$ subunits. Expression of chimeric α subunit cDNAs should prove useful for elucidating the structural basis of Na,K-ATPase function.

Na,K-ATPase is the enzyme responsible for active transport of Na⁺ and K⁺ in most animal cells. The enzyme couples hydrolysis of ATP to the movement of Na and K ions across the plasma membrane, thus producing the electrochemical gradients that are the primary source of energy for active transport of nutrients, the action potential of excitable tissues, and the regulation of cell volume (9, 14). In addition to its role as a cation transporter, Na,K-ATPase is the cellular target of the cardiac glycosides, notably ouabain and digoxin (9). Cardiac glycoside inhibition of Na,K-ATPase activity has provided a valuable tool in the study of Na,K-ATPase function and mechanism. The sensitivity of ATPase to cardiac glycosides also appears to be important for the therapeutic efficacy of this class of compounds in the treatment of congestive heart failure. However, the molecular basis for cardiac glycoside-enzyme interaction has not been clearly explained.

In all tissues from which Na,K-ATPase has been isolated, the enzyme has been shown to consist of two subunits: an α subunit of ~100 kilodaltons, which contains the binding sites for ATP, and a glycosylated β subunit with a molecular mass of ~55 kilodaltons (2). The function of the β subunit remains unknown. Ouabain is believed to interact with the α subunit on the external face of the protein (25). It is known that this drug binds to and subsequently inhibits Na,K-ATPase from a variety of sources, including monkeys (5) and humans (21), whereas the corresponding rodent enzyme is relatively insensitive to the drug (1).

Recently, cDNA clones that encode three rat α subunit isoforms (13, 26) and two rat β subunit isoforms (22, 23, 28) have been identified. Chromosomal mapping studies revealed that the three α subunit isoforms are encoded by separate genes, each of which is located on a different murine chromosome (18). Both ouabain resistance and the murine Na,K-ATPase $\alpha 1$ subunit gene have been assigned to mouse chromosome 3 (18, 20). Gene transfer experiments have shown that rat and mouse Na,K-ATPase $\alpha 1$ subunit

cDNAs confer ouabain resistance to ouabain-sensitive primate cells (5, 17). Transfer of ouabain resistance by rodent $\alpha 1$ subunit cDNA is consistent with the view that this Na,K-ATPase subunit contains a region sufficient to determine species-specific variation in ouabain sensitivity.

Several lines of evidence suggest that isoforms of the rat Na,K-ATPase α subunit also differ with respect to ouabain sensitivity. Sweadner has demonstrated differential ouabain sensitivity between the $\alpha 1$ (resistant) and $\alpha 2$ (sensitive) subunit isotypes in rat brain (27). Analysis of monkey cells made ouabain resistant by transfer of metaphase mouse chromosomes suggested that the rodent Na,K-ATPase $\alpha 2$ and $\alpha 3$ subunits are more sensitive to ouabain inhibition than is the rodent $\alpha 1$ subunit (6). Cell lines derived by transfection of rat $\alpha 3$ subunit cDNA express a component of Na,K-ATPase activity with high sensitivity to ouabain (12). However, the genetic basis for differential ouabain sensitivity among Na,K-ATPase isoforms has not been established.

We examined determinants which contribute to differential ouabain sensitivity by analyzing chimeric constructs containing different segments of ouabain-resistant rat $\alpha 1$ and ouabain-sensitive human $\alpha 1$ or rat $\alpha 2$ subunit cDNAs. By expressing these constructs in CV-1 cells, we obtained a measurement of the level of ouabain resistance of each chimera, as well as parental cDNAs. Chimeras in which the 5' end of ouabain-sensitive human $\alpha 1$ or rat $\alpha 2$ subunit cDNAs were replaced by the 5' end of ouabain-resistant rat $\alpha 1$ subunit cDNA conferred resistance to 100 μ M ouabain to CV-1 cells. Rat $\alpha 2$ subunit cDNA and a rat $\alpha 2$ -rat $\alpha 1$ cDNA chimera conferred resistance to 0.5 μ M ouabain to monkey cells. These results indicate that determinants that contribute to ouabain resistance reside within the amino-terminal portions of the rat $\alpha 1$ and $\alpha 2$ subunits. The ability to assess the biological activities of chimeric α subunit cDNA constructs should permit more precise localization of the sites responsible for differential ouabain sensitivity. This approach should also allow identification of other functional domains within Na,K-ATPase.

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MATERIALS AND METHODS

Construction of expression vectors. cDNA clones that encode the rat $\alpha 2$ and human $\alpha 1$ subunit isoforms of Na,K-ATPase were separately inserted into expression vector pSV2HS. The vector was derived from pSV2neo as follows. pSV2neo was digested with *Hind*III and *Sma*I to release a 1.3-kilobase (kb) fragment containing the *neo* gene. The 4.4-kb vector DNA segment was linked with *Hind*III (Pharmacia), religated, and propagated in *Escherichia coli* DH-1 to generate plasmid pSV2HS (5).

A cDNA clone containing the entire coding region of the rat Na,K-ATPase $\alpha 2$ subunit was constructed in pUC19 by in vitro recombination of two partial overlapping clones (4, 13) which shared a common *Msr*II site. The cDNA fragment was isolated from pUC19 by digestion with *Eco*RI, linked with *Hind*III, and inserted into the *Hind*III site of pSV2HS to yield expression vector pSV2R $\alpha 2$. The cDNA insert commences 68 base pairs upstream of the translation start site and ends 235 base pairs downstream of the translation stop site.

A full-length human $\alpha 1$ subunit cDNA was isolated from a human fetal liver λ gt11 cDNA library by using the partial human $\alpha 1$ cDNA clone pHANK (3) as a probe. Sequencing of the clone revealed 100% identity with the sequence previously reported for human $\alpha 1$ subunit cDNA (16). The cDNA fragment was isolated from λ gt11 by digestion with *Aar*I and *Hgi*AI, linked with *Hind*III, and inserted into pSV2HS to yield expression vector pSV2H $\alpha 1$. The human $\alpha 1$ subunit cDNA insert commences 8 base pairs upstream of the translation start site and ends 135 base pairs downstream of the translation stop site. Details concerning expression vector pSV2R $\alpha 1$, which contains a cDNA that encodes the entire rat Na,K-ATPase $\alpha 1$ subunit, have been described previously (5).

Construction of chimeric cDNAs. Chimeric constructs between rat $\alpha 1$ subunit cDNA and human $\alpha 1$ or rat $\alpha 2$ subunit cDNA were designed so that junction sites occurred at identical residues within each of the α subunit proteins. A rat $\alpha 1$ -rat $\alpha 2$ chimera (RAT1RAT2-N) was constructed by ligation of a 2.2-kb *Hind*III-*Nco*I fragment derived from the 5' end of rat $\alpha 1$ cDNA to a 1.1-kb *Nco*I-*Hind*III fragment derived from the 3' end of rat $\alpha 2$ cDNA. A rat $\alpha 1$ -human $\alpha 1$ chimera (RAT1HUM1-H) was constructed by ligation of a 1.55-kb *Hind*III-*Bam*HI fragment derived from the 5' end of rat $\alpha 1$ subunit cDNA to a 1.7-kb *Bam*HI-*Hind*III fragment derived from the 3' end of human $\alpha 1$ subunit cDNA. The reciprocal chimera (HUM1RAT1-H) was constructed by ligation of a 1.56-kb *Hind*III-*Bam*HI fragment derived from the 5' end of human $\alpha 1$ cDNA to a 1.7-kb *Bam*HI-*Hind*III fragment derived from the 3' end of rat $\alpha 1$ cDNA. A rat $\alpha 1$ -rat $\alpha 2$ chimera (RAT1RAT2-B) was obtained by ligation of a 1.0-kb *Hind*III-*Bal*I fragment derived from the 5' end of rat $\alpha 1$ cDNA to a 2.3-kb *Bal*I-*Hind*III fragment derived from the 3' end of rat $\alpha 2$ subunit cDNA. The reciprocal construct (RAT2RAT1-B) was made by ligation of a 1.1-kb *Hind*III-*Bal*I fragment derived from the 5' end of rat $\alpha 2$ cDNA to a 2.2-kb *Bal*I-*Hind*III fragment derived from the 3' end of rat $\alpha 1$ cDNA. A rat $\alpha 1$ -human $\alpha 1$ chimera (RAT1HUM1-A) was obtained by ligation of a 0.53-kb *Hind*III-*Asu*II fragment derived from the 5' end of rat $\alpha 1$ cDNA to a 2.7-kb *Asu*II-*Hind*III fragment derived from the 3' end of human $\alpha 1$ subunit cDNA. The reciprocal construct (HUM1RAT1-A) was obtained by ligation of a 0.52-kb *Hind*III-*Asu*II fragment from the 5' end of human $\alpha 1$ subunit cDNA to a 2.7-kb

*Asu*II-*Hind*III fragment derived from the 3' end of rat $\alpha 1$ subunit cDNA.

Cell culture and DNA transfection. African green monkey CV-1 cells were grown in Dulbecco modified Eagle medium supplemented with 10% fetal bovine serum (Armour). Plasmid DNA was transfected into CV-1 cells by using the calcium phosphate coprecipitation technique as previously described (5, 17). Two days after transfection, CV-1 cells were split 1:4 and selected with medium containing ouabain (1 μ M). Plasmids containing full-length rat $\alpha 2$, human $\alpha 1$, or RAT2RAT1-B chimeric cDNA sequences were each co-transfected into CV-1 cells at a molar ratio of 10:1 with pSV2neo. Transfectants were selected with G418 (0.5 mg/ml), and colonies were isolated at 4 weeks. Established cell lines were maintained in medium containing 0.2 mg of G418 per ml. To assess the extent of ouabain resistance, each cell line was then subcultured free of G418 into multiwell plates in medium containing ouabain (at concentrations ranging from 10 nM to 1 μ M). Ten days after exposure to ouabain, cells were fixed with methanol, stained with Giemsa, and quantitated by using a Kodak Bio Image densitometer.

RESULTS

Identification of a region contributing to differential ouabain sensitivity. To localize sites within the Na,K-ATPase α subunit which contribute to differential ouabain sensitivity, we constructed a panel of seven chimeras between ouabain-resistant rat $\alpha 1$ and ouabain-sensitive rat $\alpha 2$ or human $\alpha 1$ subunit cDNAs. A schematic representation of the chimeras is presented in Fig. 1. All chimeric constructs give rise to cDNAs with intact open reading frames. Although the human and rat $\alpha 1$ subunits each consist of 1,018 amino acids (13, 16, 26) and the rat $\alpha 2$ subunit consists of 1,015 amino acids (26), all ligations occur at identical amino acid residues. The RAT1RAT2-N construct encodes an α subunit consisting of the amino-terminal 733 amino acids of the rat $\alpha 1$ subunit joined to the carboxyl-terminal 285 residues of the rat $\alpha 2$ subunit. RAT1HUM1-H chimeric cDNA and the reciprocal construct HUM1RAT1-H encode proteins consisting of the amino-terminal 513 residues of the rat $\alpha 1$ and human $\alpha 1$ subunits, respectively. The RAT1RAT2-B construct codes for a protein consisting of the amino-terminal 337 amino acids of the rat $\alpha 1$ subunit joined to the carboxyl-terminal 680 amino acids of the rat $\alpha 2$ subunit. The reciprocal chimera, RAT2RAT1-B, codes for an α subunit consisting of the amino-terminal 335 residues of the rat $\alpha 2$ subunit joined to the carboxyl-terminal 681 residues of the rat $\alpha 1$ subunit. The RAT1HUM1-A chimera and the reciprocal construct, HUM1RAT1-A, encode proteins consisting of the amino-terminal 172 residues of the rat $\alpha 1$ or human $\alpha 1$ subunit, respectively.

Each of the chimeric constructs was separately inserted into expression vector pSV2HS. Plasmids were transfected into CV-1 cells by the calcium phosphate coprecipitation procedure (5, 17), and transfected cells were selected for the ability to proliferate in 1 μ M ouabain. The results of this experiment are shown in Table 1. In three separate experiments, transfection of pSV2R $\alpha 1$ into CV-1 cells gave an average of 92 ouabain-resistant colonies per 10^6 cells per μ g of DNA. No ouabain-resistant colonies were produced by transfection of CV-1 cells with plasmids containing human $\alpha 1$ or rat $\alpha 2$ cDNA or in control groups that were transfected with pSV2neo or mock transfected. Introduction of the RAT1RAT2-N chimera into CV-1 cells gave 100 ouabain-resistant colonies per 10^6 cells per μ g of DNA (one experi-

FIG. 1. Schematic Drawing of Chimeric Na,K-ATPase α subunit cDNAs. (A) Predicted secondary structure of the Na,K-ATPase α subunit (13). NH₂ and COOH refer to the amino- and carboxyl-terminal ends of the protein, respectively. There are seven putative membrane-spanning domains (H1 through H7). The restriction endonuclease sites used for constructions are denoted by single letters: A, *AsuII*; B, *BallI*; H, *BamHI*; N, *NcoI*. (B) Chimeric α subunit cDNAs. Thick lines represent rat α 1 subunit cDNA sequences, thin lines represent human α 1 subunit cDNA sequences, and dotted lines represent rat α 2 subunit cDNA sequences. Restriction endonuclease junction sites are shown above each construct. The 5' end of each cDNA is at the left, and the 3' end is at the right.

ment). Transfection of the RAT1HUM1-H, RAT1RAT2-B, and RAT1HUM1-A chimeras gave an average of 80, 46, and 74 ouabain-resistant colonies, respectively. No ouabain-resistant colonies were found in cells transfected with any of the reciprocal chimeric constructs. These results suggest that sequences which make a major contribution to ouabain resistance reside in the 5' end of Na,K-ATPase α 1 subunit cDNA.

To assess the extent of ouabain resistance in transfectants containing chimeric α subunit cDNAs, we measured the survival of cell lines designated R1R2-N, R1H1-H, R1R2-B, R1H1-A (RAT1RAT2-N, RAT1HUM1-H, RAT1RAT2-B, and RAT1HUM1-A transfectants, respectively), and R1 (rat α 1 transfectant); NIH 3T3 cells; and recipient CV-1 cells at various concentrations of ouabain. Duplicate 1.0-ml cultures containing $\sim 10,000$ cells were plated in 24-well plates in Dulbecco modified Eagle medium–10% fetal bovine serum with 10 nM, 100 nM, 1 μ M, 10 μ M, or 100 μ M ouabain. After 10 days of incubation, cells were fixed in methanol and

TABLE 1. Efficiency of transfection of Na,K-ATPase chimeric α subunit cDNA clones into CV-1 cells^a

cDNA transfected	Colonies/ μ g of DNA per 10^6 cells		
	Expt 1	Expt 2	Expt 3
pSV2R α 1	58	69	151
RAT1RAT2-N	ND	100	ND
RAT1HUM1-H	74	80	88
HUM1RAT1-H	0	0	0
RAT1RAT2-B	65	33	41
RAT2RAT1-B	0	0	0
RAT1HUM1-A	80	55	87
HUM1RAT1-A	0	0	0
pSV2H α 1	0	0	0
pSV2R α 2	0	0	0
pSV2neo	0	0	0
None (mock transfection)	0	0	0

^a CV-1 cells were transfected with the α subunit cDNA constructs listed. At 48 h after transfection, the cells were subcultured at a dilution of 1:4. Ouabain was added 8 h later to a final concentration of 1 μ M. At 4 weeks after transfection, the colonies were fixed, stained with Giemsa, and quantitated by densitometry. ND, Not determined.

stained with Giemsa. The absorbance of stained cells in each well was measured by using a Kodak Bio Image densitometer (Fig. 2). The survival of each of the four transfectant clones at each ouabain concentration tested was the same as that of cells transfected with rat α 1 cDNA and NIH 3T3 cells. CV-1 cells were efficiently killed by ouabain at concentrations greater than 0.1 μ M. These results demonstrate that chimeric α subunit cDNAs are capable of transferring resistance to ouabain comparable to that obtained by transfection of parental rat α 1 subunit cDNA.

Rat α 2-subunit cDNA confers resistance to intermediate levels of ouabain. To determine whether cDNAs that encode the rat α 2 or human α 1 subunit of Na,K-ATPase were capable of transferring resistance to ouabain, plasmids pSV2R α 2 and pSV2H α 1 were separately cotransfected into CV-1 cells with pSV2neo. Colonies of G418-resistant transfectants were picked at 4 weeks and expanded into cell lines. Each cell line was then subcloned in multiwell plates (without G418) and grown in concentrations of ouabain ranging from 10 nM to 1 μ M. The results of this experiment are shown in Fig. 3. Cell line R1, transfected with pSV2R α 1, survived at ouabain concentrations as high as 100 μ M. CV-1 cells did not survive at concentrations of ouabain above 0.1 μ M. The survival of each of the pSV2H α 1 cotransfectants was the same as that of parental CV-1 cells (data not shown). However, 4 of the 12 G418-resistant clones cotransfected with pSV2R α 2 survived exposure to levels of ouabain as high as 0.2 μ M (data not shown). One of these cell lines (α 2.1) appeared to survive in 0.5 μ M ouabain (Fig. 3). These results suggest that rat Na,K-ATPase α 2 subunit cDNA confers resistant to intermediate levels of ouabain to CV-1 cells.

To identify regions with the rat α 2 subunit which contribute to ouabain resistance, we tested the survival of cell lines cotransfected with pSV2neo and the RAT2RAT1-B chimera at various concentrations of ouabain. Twelve G418-resistant

FIG. 2. Analysis of the ouabain resistance phenotypes of cell lines transfected with chimeric α subunit cDNAs. CV-1 cells were transfected with chimeric α subunit cDNAs as described in Materials and Methods. Colonies resistant to 1 μ M ouabain were picked and expanded into mass cultures. Duplicate 1.0-ml cultures containing $\sim 10,000$ cells were plated in 24-well plates and exposed to ouabain concentrations ranging from 10 nM to 1.0 μ M. Cells were scored for survival at 10 days. The cell lines tested were R1R2-N ($\blacktriangle$), R1H1-H ($\blacktriangledown$), R1R2-B ($\blacksquare$), R1H1-A ($\bullet$), R1 ($\square$), NIH 3T3 ($\triangle$), and CV-1 ($\circ$). Each point is an average of two cultures expressed as a percentage of control CV-1 cells which received no drug.

clones were selected and expanded for further analysis, and four survived exposure to 0.2 μ M ouabain (data not shown). One of the cell lines, designated $\alpha 2/1.6$, survived in 0.5 μ M ouabain (Fig. 3). These results suggest that determinants responsible for ouabain resistance also reside in the 5' end of rat $\alpha 2$ subunit cDNA.

DISCUSSION

We have demonstrated the utility of expressing chimeric Na,K-ATPase α subunit cDNAs for systematic study of the structure-function relationships for this gene family. The level of ouabain resistance derived by expression of chimeric α subunit cDNAs compared well to the level of ouabain resistance derived by expression of each of the parental α subunit cDNAs. This method, therefore, provides an accurate approach to the analysis of sites within the α subunit that contribute to differential ouabain sensitivity.

There are two alternative models of the basis for differential ouabain sensitivity between Na,K-ATPase α subunit isoforms. In one model, ouabain sensitivity may be determined by a number of independent elements within each α subunit isoform. In this model, chimeras between ouabain-resistant and ouabain-sensitive α subunits in which such elements are moved from one context to another should produce α subunits intermediate in ouabain sensitivity between the two parent α subunits. The results presented here are not consistent with this view, since intermediate levels of

FIG. 3. Ouabain resistance phenotypes of cotransfectant clones $\alpha 2.1$ ($\bullet$) and $\alpha 2/1.6$ ($\blacksquare$), clone R1 ($\square$), and recipient line CV-1 ($\circ$). CV-1 cells were cotransfected with plasmids pSV2neo and pSV2R $\alpha 2$ or the RAT2RAT1-B chimera. G418-resistant colonies were picked at 4 weeks and expanded into cell lines. Duplicate 1.0-ml subcultures (containing $\sim 10,000$ cells) were plated in 24-well plates, exposed to ouabain concentrations ranging from 10 nM to 1 μ M, and scored for survival at 10 days. Each point is an average of two cultures expressed as a percentage of control CV-1 cells which received no drug.

ouabain sensitivity were not found in any of the chimeras tested. The second possibility is that a single element, or a number of closely linked elements, determines the ouabain sensitivity of an α subunit isoform. In this model, chimeric α subunit cDNAs should produce α subunits with the ouabain sensitivity of one parent α subunit or the other. Our results are most consistent with this possibility.

Previous studies using photoaffinity and fluorescent derivatives of ouabain have suggested that interaction of ouabain with Na,K-ATPase is complex and may involve extracellular domains from both the amino- and carboxyl-terminal regions of the α subunit (7, 8, 10, 15, 19, 25). Possible involvement of the β subunit in cardiac glycoside-enzyme interaction has also been described (11). While several regions of Na,K-ATPase may interact with ouabain, our data indicate that a sequence(s) within the amino-terminal portion of the $\alpha 1$ subunit determines the ouabain sensitivity of this Na,K-ATPase isoform. Transfer of ouabain resistance by chimeric cDNAs in which segments of the 5' end of the human $\alpha 1$ or rat $\alpha 2$ subunit were replaced by equivalent segments of the 5' end of the rat $\alpha 1$ subunit indicate that sequences which make a major contribution to ouabain resistance reside within the first 172 residues of the rat $\alpha 1$ subunit. These results are consistent with the data obtained by Price and Lingrel (24), who identified two residues within the H1-H2 extracellular domain of the sheep $\alpha 1$ subunit, Gln-111 and Asn-122, which appear to determine the ouabain sensitivity of this Na,K-ATPase isoform.

Our results also support the presence of sequences that contribute to ouabain resistance in the 5' end of rat $\alpha 2$

subunit cDNA. We found that rat $\alpha 2$ subunit cDNA was capable of transferring resistance to 0.5 μ M ouabain to CV-1 cells. This indicates that the rat Na,K-ATPase $\alpha 2$ subunit is ~500-fold more sensitive to ouabain than is the rat $\alpha 1$ subunit but ~20-fold more resistant to ouabain than is any of the primate α subunit isoforms. Further, the RAT2RAT1-B chimera also conferred resistance to 0.5 μ M ouabain to monkey cells. This suggests that determinants of ouabain resistance reside within the amino-terminal portion of the rat $\alpha 2$ isoform. The RAT2RAT1-B chimera contains two extracellular domains, H1-H2 and H3-H4, which are contributed by rat $\alpha 2$ subunit cDNA. It is therefore possible that either or both of these domains determine the ouabain-resistant character of the rat $\alpha 2$ subunit.

Our data are consistent with the view that the human $\alpha 1$ subunit is as sensitive to ouabain inhibition as is the monkey $\alpha 1$ subunit. It is not possible, therefore, to distinguish whether the failure to detect transfer of ouabain resistance by human $\alpha 1$ cDNA in our protocol is due to the inherent ouabain sensitivity of this isoform or to other aspects of the transfer procedure. For example, it is possible that the pSV2H $\alpha 1$ expression vector lacks biological activity. We view this explanation as unlikely, since the RAT1HUM1-A and RAT1HUM1-H chimeras (containing 83 and 50% human $\alpha 1$ subunit cDNA sequences, respectively) are biologically active. However, deletions or other artifacts in the pSV2H $\alpha 1$ vector could prevent cDNA expression. Introduction of the vector into a ouabain-resistant cell line and expression of Na,K-ATPase activity with high sensitivity to ouabain would provide definitive evidence of the biological activity of this construct.

The ability to assess the biological activities of chimeric Na,K-ATPase α subunit cDNAs permits the application of molecular genetic approaches to the analysis of structure-function relationships for the enzyme that establishes the internal Na^+ - K^+ environment of most animal cells. It is important to note in this context that all chimeras containing the 5' end of rat $\alpha 1$ subunit cDNA conferred resistance to ouabain comparable to that of naturally resistant NIH 3T3 cells. This suggests that the functional integrity of chimeric α subunits was not fundamentally altered. Our results provide an initial structural map for understanding the molecular basis of differential ouabain sensitivity between isoforms of Na,K-ATPase. This strategy should be useful for identification of other functional domains within Na,K-ATPase and defining the functional differences between α subunit isoforms.

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