The primary objective of this study was to examine the functional role of the Na,K-ATPase α1 isoform in the regulation of cardiac contractility. Previous studies using knock-out mice showed that the hearts of animals lacking one copy of the α1 or α2 isoform gene exhibit opposite phenotypes. Hearts from α2+/− animals are hypercontractile, whereas those of the α1+/− animals are hypocontractile. The cardiac phenotype of the α1+/− animals was unexpected as other studies suggest that inhibition of either isoform increases contraction. To help resolve this difference, we have used genetically engineered knock-in mice expressing a ouabain-sensitive α1 isoform and a ouabain-resistant α2 isoform of the Na,K-ATPase, and we analyzed cardiac contractility following selective inhibition of the α1 isoform by ouabain. Administration of ouabain to these animals and to isolated heart preparations selectively inhibits only the activity of the α1 isoform without affecting the activity of the α2 isoform. Low concentrations of ouabain resulted in positive cardiac inotropy in both isolated hearts and intact animals expressing the modified α1 and α2 isoforms. Pretreatment with 10 μM KB-R7943, which inhibits the reverse mode of the Na/Ca exchanger, abolished the cardiotoxic effects of ouabain in isolated wild type and knock-in hearts. Immunoprecipitation analysis demonstrated co-localization of the α1 isoform and the Na/Ca exchanger in cardiac sarcolemma. The α1 isoform co-immunoprecipitated with the Na/Ca exchanger and vice versa. These results demonstrate that the α1 isoform regulates cardiac contractility, and that both the α1 and α2 isoforms are functionally and physically coupled with the Na/Ca exchanger in heart.

Active Na⁺ transport across the cardiac sarcolemma, driven by the Na,K-ATPase, is an important regulator of cardiac function (1, 2). The intracellular Na⁺ concentration affects a number of physiological processes in cardiac myocytes, including intracellular Ca²⁺ handling, contraction-relaxation processes, pH regulation, energy metabolism, and cell growth (1–2). Alterations in the maintenance of normal intracellular Na⁺ homoestasis result in heart failure (1).

Na,K-ATPase is a heterodimer composed of α and β subunits (3). The α subunit is the catalytic subunit, and it binds translocating cations and ATP. The β subunit is also the pharmacological receptor for cardiac glycosides. These compounds inhibit Na,K-ATPase activity and are used in the treatment of congestive heart failure. There are four isoforms of the α subunit, each with a distinct tissue distribution and developmental pattern of expression, suggestive of their differential and tissue-specific functional roles (4–10). Depending on the species, different combinations of these isoforms are present in heart. The α1 and α2 isoforms are expressed in rodent heart, whereas three isoforms (α1, α2, and α3) are expressed in human heart (5, 10, 11). As multiple isoforms are expressed in heart, it is possible that they play different biological roles.

Previous studies using knock-out mice showed that the hearts of animals lacking one copy of the α1 or α2 isoform gene exhibit opposite phenotypes (6). The hearts from α2+/− animals, in which α2 levels were reduced by 50%, were hypercontractile, whereas those of the α1+/− animals, in which α1 levels were reduced by 40%, were hypocontractile. Calcium transients were increased in α2+/− cardiac myocytes but were unchanged in α1+/− myocytes, suggesting that reduced levels of the α2 isoform, but not α1 isoform, affect Ca²⁺ handling. Furthermore, administration of ouabain to the hypercontractile α1+/− hearts led to a positive inotropic response. On the basis of these data, it was proposed that the α2 isoform mediates positive cardiac inotropy, whereas the α1 isoform mediates the toxic effects of cardiac glycosides. A number of studies re-enforced the importance of the α2 isoform in the regulation of Ca²⁺ transients during contraction (7–9, 12). Also, we confirmed that the α2 isoform mediates the positive cardiac inotropy observed at low concentrations of ouabain, by comparing the effect of ouabain on cardiac contractility in wild type hearts expressing the ouabain-sensitive α2 isoform with that in hearts expressing a genetically modified, ouabain-resistant α2 isoform (13).

Several reports suggest that the α1 isoform modulates cardiac contractility differently than that proposed based on the hypocontractile cardiac phenotype in the isolated α1+/− hearts (14–18). The high expression of the α1 isoform in T-tubules of rat cardiac myocytes suggests that it has a major role in the regulation of cardiac contractility (14, 15). An increased contraction of isolated rat cardiac myocytes has been attributed to a reduction in α1 isoform expression through dominant negative interference with a fragment of the α2 isoform (16). However, in these cardiac myocytes a reduction in the expression of the α3 isoform was also detected, which could contribute to the enhanced contractility. Also a biphasic ouabain dose response in isolated rodent heart preparations is well established, and
the positive inotropic effect of high concentrations of ouabain has been accounted for by inhibition of the low affinity α1 isoenzyme (17, 18). However, as this second phase of inhibition always occurs following inhibition of the α2 isoenzyme, it is uncertain whether this pre-dispositions the heart to further contraction following inhibition of the α1 isoenzyme.

To address directly and unambiguously the role of the α1 isoenzyme, we developed animals in which the α1 isoenzyme is ouabain-sensitive and the α2 isoenzyme is ouabain-resistant, and we analyzed cardiac contractility following administration of ouabain that would inhibit only the α1 isoenzyme without altering the α2 isoenzyme. The present study demonstrates that the α1 isoenzyme regulates cardiac contractility and is functionally and physically coupled with the Na/Ca exchanger.

**EXPERIMENTAL PROCEDURES**

**Generation of a Mice Expressing the Cardiac Glycoseptide-sensitive α1 and α2 Isoforms of Na,K-ATPase**—The R111Q and D122N amino acid substitutions were introduced into the α1 isoenzyme by site-directed mutagenesis, as described previously (13). Furthermore, 2 silent base pair substitutions were made to introduce an EcoRI site. The LoxP-neomycin-LoxP cassette was cloned into intron 3 at a site 750 bp upstream of exon 4. The thymidine kinase gene was inserted downstream of the cloning sequence. The presence of the desired mutation in the targeting vector was verified by both sequence analysis and EcoRI restriction digestion. The Duffy ES cell line was transfected with the targeting vector by electroporation, and successful substitutions were introduced into the α1 isoform gene. The presence of the EcoRI site and an EcoRI site. The LoxP-neomycin-LoxP cassette was cloned into intron 3, and the 3′ probe was derived from intron 4 of the α1 isoform gene. The presence of the EcoRI site and desired mutation in exon 4 was determined in successfully targeted ES cells by PCR using the P1 (5′-AGCTCGAGGATCTGG) and the P2 (5′-CTCTAACCACCCTCCTAG) primers, and the amplified exon 4 fragment was digested with EcoRI and analyzed on a 1% agarose gel. Successfully targeted ES cells were subjected to the second electroporation with the Cre recombinase encoding vector. Following the transfection, ES clones were treated with EcoRI restriction enzyme. Successfully targeted ES cell line was transfected with the targeting vector by electroporation, and successful mutation in the targeting vector was verified by both sequence analysis and EcoRI restriction digestion. The Duffy ES cell line was transfected with the targeting vector by electroporation, and successful substitution in the targeting vector was verified by both sequence analysis and EcoRI restriction digestion. The Duffy ES cell line was transfected with the targeting vector by electroporation, and successful substitution in the targeting vector was verified by both sequence analysis and EcoRI restriction digestion.

**Western blot analysis**—Western blot analysis was performed via standard methods (13). Briefly, protein samples were incubated for 30 min at 37 °C in Laemmli Sample Buffer and separated by electrophoresis in 10% polyacrylamide gels. The separated proteins were transferred to polyvinylidene difluoride membranes and blocked in 1% non-fat dry milk in TBST (50 mM Tris (pH 7.4), 150 mM NaCl, 0.01% Tween 20) for 1 h at room temperature. The blots were incubated in TBST containing a-1 isoform-specific monoclonal antibody (αM, University of Iowa Developmental Studies Hybrid Bank), α2 isoform-specific monoclonal HERED antibody (Dr. Alicia McDonough), α3 isoform-specific monoclonal MA3–915 antibody (Affinity Bioreagents), and KETTY polyclonal antibody that recognized the C-terminal KETTY sequence of all α isoforms (Dr. Jack Kyte) at 4 °C overnight. For Western blot analysis of the immunoprecipitated proteins, additional antibodies were used as follows: mouse anti-Na/Ca exchanger, NCX (Affinity Bioreagents), mouse anti-plasma membrane Ca-ATPase 1 (Affinity Bioreagents), mouse anti-dihydropyridine receptor α2, DHPR α2 (Affinity Bioreagents). After incubation with peroxidase-conjugated goat anti-mouse IgG antibody (Jackson ImmunoResearch), immunoreactivity was visualized following treatment with the ECL Western blotting Detection Reagents (Amersham Biosciences) by using the Kodak BioMax MR x-ray film.

**Analysis of Total [3H]Ouabain Binding**—Total [3H]ouabain binding was performed as described previously (13). Briefly, 100–500 μg of crude membrane preparations were incubated at 37 °C for 6 h in a reaction mixture containing 20 μM [3H]ouabain, 10 mM sodium metavanadate, 5 mM MgCl₂, 50 mM Tris-HCl (pH 7.4), 5 mM Tris-PO₄, and either 0 or 30 μM unlabeled ouabain. Following incubation, the crude membrane preparations were filtered through the 0.2-μm glass microfiber Whatman GF/C filter paper and washed five times with 7 ml of water using a Brandel cell harvester. The filter-associated radioactivity was determined by liquid scintillation counting. Nonspecific [3H]ouabain for each microsomal preparation was determined using an excess of unlabeled ouabain (1 nM), and this value was subtracted from total binding obtained using 30 μM ouabain. Subtraction of nonspecific binding of total binding obtained in kidney samples from targeted animals resulted in a value of zero.

**Na,K-ATPase Activity**—The Na,K-ATPase activity was performed as described previously (21). Briefly, the assay medium for Na,K-ATPase activity contained 130 mM NaCl, 20 mM KCl, 50 mM choline-Cl, 3 mM MgCl₂, 3 mM ATP, 0.5 mM EDTA, 50 mM imidazole, pH 7.2 (20 °C), ~1 mM sodium azide, 10 mM NaH₂PO₄, and 0.5% (w/v) bromphenol blue) by incubation at 37 °C for 30 min.

**Analysis of Cardiac Contractility in Intact Closed-chest Animals**—The analysis in intact animals was performed as described previously (13). The mean arterial pressure was measured using a low-compliance pressure transducer (COBE Cardiovascular; Arvada, CO). A high fidelity, 1.5-French Millar Mikro-Tip transducer (model SPR-300D; Millar Instruments, TX) was inserted into the left ventricle through the femoral incision. Dobutamine and ouabain were infused through the cannulated right femoral vein. Mean arterial pressure and intraventricular pressure from the COBE transducer and the Millar transducer were analyzed using the MacLab/4s data acquisition system connected to a Macintosh 7100/80 computer. Average values for heart rate, mean arterial pressure, and systolic left ventricular pressure were measured from the pressure waveforms and were determined for each animal from at least 50 consecutive beats during the final 30 s of each 3-min dosage period. Maximum dP/dt and dP/dt at 40 mM Hg (dP/dt max) for

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The abbreviations used are: DHPR, dihydropyridine receptor; ES, embryonic stem cell; NCX, Na/Ca exchanger; PMCA 1, plasma membrane Ca²⁺-ATPase 1.
**RESULTS**

**Generation of Mice Expressing a Cardiac Glycoside-sensitive \(a_1\) Isoform of Na,K-ATPase**—The ouabain sensitivity of the mouse \(a_1\) isoform was increased by introducing R111Q and D122N amino acid substitutions into the first extracellular domain of this isoform (Fig. 1). The Gln-111 and Asn-122 residues are naturally present in the high affinity human and sheep \(a_1\) isoforms and were shown previously to confer sensitivity to cardiac glycosides (23, 24). The R111Q and D122N substitutions were introduced by 2-bp mutations in exon 4 of the \(a_1^{\text{10S}}\) \(a_2^{\text{0S}}\) isoforms. Introduced R111Q and D122N amino acid substitutions and an EcoRI site were retained in genomic DNA of the \(a_1^{\text{10S}}\) \(a_2^{\text{0S}}\) animals.

Chimeric animals were generated by injecting the targeted ES cell clone into blastocysts that were then implanted into pseudopregnant SVJ/129 mice. The chimeric offspring were

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**Fig. 1. Generation of mice expressing the cardiac glycoside-insensitive \(a_1\) Na,K-ATPase (\(a_1^{\text{10S}}\) \(a_2^{\text{0S}}\)).** A, introduction of the R111Q and D122N substitutions in the mouse \(a_1\) isoform of Na,K-ATPase. Two base pair mutations were introduced into exon 4 by PCR amplification, resulting in the R111Q and D122N amino acid substitutions. Two silent base pair mutations were also introduced to form a unique EcoRI site. B, Cre-LoxP targeting strategy. Top, a restriction map of the region in one of the wild type alleles that would be involved in homologous recombination. Middle, targeting vector and a schematic of the targeted allele following successful homologous recombination. Bottom, targeted allele following successful excision of the neomycin cassette by Cre-recombinase. The 5’ and 3’ probes were used for screening of targeted ES cells. Primers P1 and P2 were used in PCR amplification of exon 4 for verification of EcoRI site presence. Primers P3 and P4 were used for genotyping of the offspring animals. C, sequence histogram of the partial exon 4 sequence from the \(a_1^{\text{10S}}\) \(a_2^{\text{0S}}\) animals. Introduced R111Q and D122N amino acid substitutions and an EcoRI site were retained in genomic DNA of the \(a_1^{\text{10S}}\) \(a_2^{\text{0S}}\) animals.
crossed to Black Swiss females to yield heterozygous α1S/α2R/R animals. Through mating of heterozygous mice, wild type (α1R/R α2R/R), heterozygous (α1S/α2S/S), and homozygous (α1S/α2S/S) offspring were generated. These genotypes were born in expected Mendelian ratios. The α1S/α2S/S animals retained the desired R111Q and D122N substitutions as shown by the sequence histogram of their genomic DNA (Fig. 1C).

Generation of the α1S/S, α2R/R Mice—Animals expressing the ouabain-sensitive α1 isoform and ouabain-resistant α2 isoform (α1S/α2R/R) were developed by mating ouabain-sensitive α1 isoform mice (α1S/α2S/S) with the ouabain-resistant α2 isoform (α2R/R) mice (13). Although the α2 isoform is resistant to ouabain in double-knock-in animals, i.e. α1S/α2R/R mice, more ouabain binding was detected in heart preparations from α1S/α2R/R mice than from wild type. This reflects the increase in ouabain sensitivity of the α1 isoform and indicates that this isoform contributes more to the total levels of the Na,K-ATPase than the α2 isoform.

By comparing the total ouabain binding in skeletal muscle and heart between the wild type and targeted mice, we determined the relative abundance of the α1 and α2 isoforms in these tissues. This is possible as in these tissues the total ouabain binding is contributed by the α2 isoform in wild type mice and by the α1 isoform in targeted mice. There was four times more ouabain bound in wild type skeletal muscle than in skeletal muscle from α1S/α2R/R mice. Therefore, the α1 isoform comprises 20% and the α2 isoform 80% of total Na,K-ATPase in skeletal muscle. In contrast, there was 20 times more ouabain bound in α1S/α2R/R heart than in wild type α1R/R α2S/S. Hence, the α1 isoform comprises 95.2% and the α2 isoform comprises 4.8% of total Na,K-ATPase in heart.

Western blot analysis of the tissue from the wild type and α1S/α2R/R mice demonstrated that the expression and tissue...
distribution of the α1 and α3 isoforms are normal in targeted animals (Fig. 2C). Although the tissue distribution of the α2 isoform was normal, there was a slight, but not significant, up-regulation in its expression in targeted heart preparations. This may be due to variability in the genetic background of these animals, as expression of the α2 isoform is normal in hearts from α1S/S α2R/R (13) and α1S/S α2RΔS (data not shown) mice. However, this slight alteration in the expression of the modified/ouabain-resistant α2 isoform could have not affected the analysis because we determined the difference in the contractile parameters before and after selective inhibition of only the ouabain-sensitive α1 isoform.

To determine whether modification of ouabain sensitivity altered enzymatic activity of the α1 isoform, we analyzed the total ouabain inhabitable Na,K-ATPase activity in kidney preparations from α1S/S α2RΔS and wild type mice. There was no difference in total Na,K-ATPase activity between the two genotypes (data not shown). This was expected as amino acids Leu-111 and Asn-122 are naturally present in the human α1 isoform that is sensitive to ouabain. A 100-fold increase in sensitivity of the modified/ouabain-resistant α2 isoform could have not affected the analysis because we determined the difference in the contractile parameters before and after selective inhibition of only the ouabain-sensitive α1 isoform.

Basal Hemodynamic Parameters Are Normal in Intact Closed-chest α1S/S α2R/R Mice—Basal cardiovascular function in wild type and α1S/S α2RΔS mice was evaluated in vivo by cardiac catheterization (Table I). There were no significant differences in basal heart rate, mean arterial pressure, systolic blood pressure, left ventricular pressure, maximum rate of force of contraction, minimum rate of force of contraction, and the rate of force of contraction at 40 mm Hg between the two genotypes. This demonstrates normal physiological hemodynamics and heart function in targeted mice. This also indicates that slight down expression of the α2 isoform has not altered normal hemodynamics.

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\text{α1}^{\text{S/S}} \text{ α2}^{\text{R/R}} \text{Mice—To determine whether modification of the α1 and α2 isoforms of Na,K-ATPase alerted the normal Ca}^{2+} \text{ handling in heart, we analyzed cardiac contractility in intact wild type and α1S/S α2R/R mice following administration of dobutamine, a β-adrenergic agonist. There were no significant differences in dP/dt max, dP/dt min, and dP/dt 40 following the β-adrenergic stimuli between the two genotypes (Table I). This indicated that cardiac contraction and its response to the β-adrenergic stimuli are normal in targeted animals.}
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**Inhibition of the α1 Isoform of Na,K-ATPase Results in Positive Cardiac Inotropy**—The effect of ouabain on contractility in isolated work-performing α1S/S α2RΔS hearts was analyzed to determine the consequence of selective inhibition of the α1 isoform on cardiac contractility. Administration of ouabain increased the maximum rate of heart contraction (+dP/dt) and relaxation

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\text{A} \text{ and B)}
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and α1SS α2RR (Fig. 5, C and D) isolated hearts. Thus, the reverse mode of the Na/Ca exchanger is necessary for contraction to occur following inhibition of the α1 isoform in α1SS α2RR hearts and the α2 isoform in wild type hearts.

The α1 Isoform and Na/Ca Exchanger Form a Protein-Protein Complex in the Heart Plasma Membrane—We next deter-
mained whether the α1 isoform of the Na,K-ATPase forms a protein complex with the Na/Ca exchanger in heart plasma membranes. Previous immunoprecipitation analysis from brain demonstrated that only the α2 isoform forms a complex with the Na/Ca exchanger (20). The immunoprecipitates of the Na/Ca exchanger (NCX) and the α1 isoform were prepared from the detergent-soluble fraction of mouse heart plasma membranes. In addition, we prepared immunoprecipitates of the α2 isoform as a positive control. As a negative control, the immunoprecipitates of mouse and rabbit IgG were also prepared. The immunoprecipitation with NCX-specific antibody removed all the Na/Ca exchangers from the detergent-soluble fraction of heart membranes. As shown in Fig. 6A, a predominant NCX band of 140 kDa was detected in the precipitated fraction, and no band was detected in the post-immunoprecipitated, detergent-soluble fraction. No NCX immunoprecipitated with mouse IgG: Both the α1 and α2 isoforms of Na,K-ATPase co-immunoprecipitated to a significant extent with NCX. Most of the α2 isoform was bound by NCX, and thereby a majority of it was recovered in the immunoprecipitate. Although a significant amount of the α1 isoform co-immunoprecipitated with NCX, some of it was recovered in the post-immunoprecipitated soluble fraction. The negligible amounts of the dihydropyridine receptor, DHPR α2, co-immunoprecipitated with NCX. In contrast, the plasma membrane Ca$^{2+}$-ATPase 1 (PMCA 1) did not co-immunoprecipitate with NCX. The immunoprecipitation with mouse IgG did not remove any of these plasma membrane proteins from the detergent fraction, and thereby none was recovered in the immunoprecipitate.

As shown in Fig. 6B, immunoprecipitation with the α1 isoform-specific antibody removed all the α1 isoform from the detergent-soluble fraction, and all the α1 isoform was recovered in the immunoprecipitate. A control immunoprecipitation with mouse IgG did not remove any of the α1 isoform. The NCX co-immunoprecipitated with the α1 isoform. However, not all NCX was bound by the α1 isoform, and some was recovered in the post-immunoprecipitated supernatant. The α2 isoform, DHPR α2, and PMCA 1 did not co-immunoprecipitate with the α1 isoform, and these proteins were recovered in the post-immunoprecipitated supernatant. The nonspecific immunoprecipitation with mouse IgG did not pull down any of the analyzed proteins and was recovered in the IgG immunoprecipitate.

The immunoprecipitation with the α2 isoform-specific antibody removed a significant amount of the α2 isoform from the solubilized heart membranes (Fig. 6C). However, some of the α2 isoform was recovered in the post-immunoprecipitated supernatant. The immunoprecipitation with rabbit IgG did not remove the α2 isoform. As expected, NCX co-immunoprecipitated with the α2 isoform. Small amounts of the NCX were bound by this isoform, and the majority of NCX was recovered in the post-immunoprecipitated supernatant. The α2 isoform did not co-immunoprecipitate with the α2 isoform. Similarly, both DHPR α2 and PMCA 1 were fully recovered in the post-immunoprecipitated supernatant. The immunoprecipitate of rabbit IgG did not contain any of the analyzed proteins.

In summary, both the α1 and α2 isoforms of Na,K-ATPase co-localized with the Na/Ca exchanger in cardiac sarcolemma. However, protein complexes formed between the α1 isoform and the Na/Ca exchanger are distinct from the Na/Ca exchanger complexes containing the α2 isoform.

**DISCUSSION**

Studies in mice lacking one copy of the α1 or α2 isoform gene suggested that these two α isoforms of Na,K-ATPase play a differential role in the regulation of cardiac contractility (6). The hearts from α2$^{−/−}$ animals are hypercontractile, whereas those of the α1$^{+/−}$ animals are hypocontractile. Although this
was expected for the α2 isoform, it was quite surprising for the α1 isoform, as several studies (14–18) suggested that reduction in the activity of the α1 isoform results in positive cardiac inotropy. We have used an approach that makes it possible to examine the functional role of each isoform of Na,K-ATPase separately. We have confirmed that the α2 isoform of Na,K-ATPase is coupled to cardiac contraction and mediates ouabain-induced positive inotropy in mouse heart. This was accomplished by comparing the effect of ouabain on ouabain-sensitive (wild type) and ouabain-resistant α2 isoform hearts (13). In the present study, we directly examined the functional role of the α1 isoform of Na,K-ATPase in the regulation of cardiac contractility by using genetically engineered animals where the α1 isoform was modified to a ouabain-sensitive form, and the α2 isoform was modified to a ouabain-resistant one. This enabled us to selectively inhibit only the α1 isoform without altering the activity of the α2 isoform. Hence, any alteration in cardiac contractility in these knock-in mice, following administration of ouabain, can only be accounted for by the reduction in the activity of the α1 isoform. This approach bypassed the embryonic lethality associated with complete genetic elimination of the α1 isoform (6). In addition, as cardiac contractility was analyzed immediately after administration of ouabain, we avoided the occurrence of genetic compensatory mechanisms that may accompany a long term down-regulation of the α1 isoform and mask the actual cardiac phenotype. Taken together, this represents a novel approach for analyzing the functional role of a specific Na,K-ATPase isozyme.

In the present study we demonstrate that a reduction in α1 isoform activity enhances cardiac contractility. This is in agreement with previous studies attributing the increased contractility of isolated rat cardiac myocytes to reduced activity of the α1 isoform (16). In addition we demonstrate that the extent of the reduction in α1 isoform activity determines whether cardiac contractility will be enhanced or compromised. Whereas inhibition of modest amounts of the α1 isoform activity at low concentrations of ouabain increased cardiac contractility, cardiac failure occurred following significant reduction in the α1 isoform activity at high concentrations of ouabain. This is expected if we consider the amounts of the α1 isoform in heart. In agreement with previous findings, our study demonstrates that the α1 isoform comprises a majority of the Na,K-ATPase in mouse heart (26, 27). Thus, it is expected that inhibition of significant amounts of Na,K-ATPase activity would result in cardiac failure. This may explain why mice lacking one copy of the α1 isoform gene have depressed cardiac contractility.

The present data suggest that the α1 and α2 isoforms play a similar role in the regulation of cardiac contractility. It is quite clear that inhibition of either the α1 or α2 isoform enhances cardiac contraction. This is of importance as human hearts express essentially equal amounts of three α isoforms (α1, α2, and α3), which are all sensitive to ouabain (10, 28). Thus, in patients with congestive heart failure, inhibition of the α1 isoform along with the α2 isoform by cardiac glycosides will contribute to increased heart contraction. In addition, the α1 isoform is not a major contributor to the total Na,K-ATPase in human heart, and it is unlikely that a significant reduction in its activity will result in cardiac failure as it does in mouse hearts. Thus, the present data should help in understanding the role of the α1 isoform in failing human hearts, where a significant down-regulation of the α1 isoform occurs (29–32).

The present study also demonstrates that the α1 isoform
regulates cardiac contractility through the reverse mode of the Na/Ca exchanger as does the α2 isoform. This is in contrast to previous studies suggesting that only the α2 isoform of Na,K-ATPase regulates the flux mode of the Na/Ca exchanger (6, 12). However, these studies did not directly analyze the role of the α1 isoform in regulating cardiac contractility and the Na/Ca exchanger transport mode. Nevertheless, our data show that the α1 isoform, as well as the α2 isoform, regulates cardiac contractility through the Na/Ca exchanger.

The current study also demonstrates that in cardiac sarclemma both the α1 and α2 isoforms form distinct protein complexes with the Na/Ca exchanger. In contrast, a recent study demonstrated that in brain the Na/Ca exchanger formed a complex with the α2 isoform and not the α1 isoform of Na,K-ATPase (20). Thus, it is possible that the functional role of the α1 isoform in brain may be different from that in contractile tissue, such as heart.

In summary, the present study represents a direct analysis of the functional role of the α1 isoform of Na,K-ATPase. We used a novel approach that allowed us to bypass the embryonic lethality associated with the complete genetic elimination of the α1 isoform and to directly analyze the role of the α1 isoform in the regulation of cardiac function. We demonstrate that the α1 isoform of Na,K-ATPase regulates cardiac contractility through the reverse mode of the Na/Ca exchanger, as does the α2 isoform. This finding demonstrates that the reversal of the Na/Ca exchanger occurs subsequent to the inhibition of the α1 isoform of Na,K-ATPase and results in increased cardiac contractility. This is in agreement with the previous analysis of the ouabain-sensitive (wild type) and ouabain-resistant α2 isoform hearts (13). Thus, inhibition of either the α1 or α2 isoform enhances cardiac contractility. Hence, the present data argue against the α2 isoform being the only Na,K-ATPase isoform regulating cardiac contractility. Although our study demonstrates that both the α1 and α2 isoforms have the same role in heart, our data cannot address whether there are subtle differences in mechanisms underlining the function of these two α isoforms in this tissue.

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The α1 Isoform of Na,K-ATPase Regulates Cardiac Contractility and Functionally Interacts and Co-localizes with the Na/Ca Exchanger in Heart
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