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Site-Specific Antibody of (Na^+ + K^+)-ATPase Augments Cardiac Myocyte Contraction without Inactivating Enzyme Activity

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(Na^+ + K^+)-ATPase regulates both excitability and contractility of the heart. Little is known about the molecular basis of the enzyme that underlies its cardiac regulatory functions. Here we demonstrate that the $_{833}$KRQPRNPKTDKLVNE$_{847}$ region, which resides in the α-subunit of rat (Na^+ + K^+)-ATPase, directly participates in the regulation of cardiac contraction. A site-specific antibody (SSA95) against this peptide sequence markedly increased intracellular Ca^{2+} transients and contraction (EC_{50} = 11.4 nM) in intact rat heart cells without inactivating the (Na^+ + K^+)-ATPase. These novel findings establish the first link between a precise structural region of the (Na^+ + K^+)-ATPase and cardiac positive inotropy.

Key Words: **(Na^+ + K^+)-ATPase; site-specific antibody; Ca^{2+} transients; positive inotropic effect; digitalis glycosides.**

(Na^+ + K^+)-ATPase (1) catalyzes the vectorial transport of Na^+ and K^+ ions across the plasma membrane of all animal cells (2) and regulates both excitation and contraction of the heart (3–5). Since William Withering used foxglove in the treatment of chronic congestive heart failure in 1785, the (Na^+ + K^+)-ATPase has been established over the years as a target receptor for the biological actions of digitalis glycosides (6–9). Although extensive efforts have been made to determine the mechanisms of the digitalis glycosides-induced positive inotropic action, the relationship between molecular sequence of the (Na^+ + K^+)-ATPase and the positive inotropic effect are not completely understood. In this study, we provide critical information and a novel approach to identify a structural region of the (Na^+ + K^+)-ATPase that is directly involved in regulation of cardiac contractility. We have generated a site-specific antibody (SSA95) against a known sequence of the (Na^+ + K^+)-ATPase. The binding of SSA95 to α-subunit of the (Na^+ + K^+)-ATPase significantly increased the Ca^{2+} transients and contraction of the rat cardiac mocytes without inactivating the activity of the enzyme. These important findings provide the first link between the structure of the (Na^+ + K^+)-ATPase and cardiac positive inotropy, thereby uncover a novel mode for (Na^++ K^+)-ATPase to regulate cardiac function.

MATERIALS AND METHODS

Materials. All reagents were purchased from Sigma Chemical Co., unless specified. [21,22-^{3}H]ouabain (15–50 Ci/mM) was from Amersham Pharmacia Biotech. Highly purified dog kidney (Na^+ + K^+)-ATPase was a gift from Dr. Jack Kyte.

Antibody preparation. The KRQPRNPKTDKLVNE peptide was synthesized according to the protein sequence reported (10–12). The polyclonal SSA95 antibody was generated in New Zealand White rabbits using KLH as a peptide carrier (Genemed). The immunoglobulins (IgG) were purified through an affinity column directed against the same synthetic peptide of the (Na^+ + K^+)-ATPase. Purified SSA95 recognizes both denatured (by Western blots) and native (Na^+ + K^+)-ATPase (by immunocytostaining). Synthetic peptide was also utilized as the specific peptide blockers for the antibodies.

Measurement of cell contraction. Cardiac myocytes were isolated from adult Sprague–Dawley rats, using a standard enzymatic method described previously (13). Isolated myocytes were suspended in a buffer containing (in mM) 137 NaCl, 5.4 KCl, 15 dextrose, 1.3 $MgSO_4$, 1.2 NaH_2PO_4, 1 $CaCl_2$, and 20 Hepes, pH 7.4. To measure cell contractility, cardiac myocytes were placed on an inverted microscope (Zeiss Model IM-35), bathed with a Hepes-buffered solution, and electrically stimulated under 0.5 Hz at room temperature. The designated reagents were added when the baseline contraction was stabilized after 10–15 min constant pacing. Cell length was monitored from the bright-field image (650 nm to 750 nm red light illumination) by an optical edge-tracking method using a photodiode array (Model 1024 SAQ, Reticon) with a 3-ms time resolution. The

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contraction amplitude was indexed by the percentage shortening of cell length.

Confocal Ca^{2+} imaging. Intracellular loading of the Ca^{2+} indicator fluo-4 was achieved by a 10-min incubation of the myocytes with 10 μM fluo-4 AM (Molecular Probes). Confocal linescan images of intracellular Ca^{2+} and cell length were acquired using a Zeiss LSM-410 confocal microscope (Carl Zeiss) equipped with an argon laser (488 nm) and a Plan-Neofluar 40×, NA 1.3, oil-immersion objective. The scan line was oriented along the long axis of the myocyte, avoiding nuclei of the cells; the scan rate and spatial resolutions were 2.0 ms/line, and 0.4 × 0.4 × 1.0 (xyz) μm^3, respectively. IDL software (Research Systems, Boulder Co.) was used for image processing, data analysis, and presentation (14). Ca^{2+} transients were calculated using the formula: $[Ca^{2+}] = k_d R/\{(k_d/[Ca^{2+}]_{rest} + 1) - R\}$, where $R = F/F_o$, the resting Ca^{2+} concentration $[Ca^{2+}]_{rest} = 100$ nM, and the dissociation constant $k_d = 1.1$ μM.

Isolation of sarcolemmal vesicles and purification of ($Na^+ + K^+$)-ATPase. Rat cardiac sarcolemmal (SL) vesicles were isolated from rat heart muscle by sucrose flotation method (15). The vesicles were tested with saponin and were predominately right-side-out in orientation (data not shown). ($Na^+ + K^+$)-ATPase was purified as described previously (16). Briefly, the SL vesicles (4.4 mg/ml) were titrated with 0.58 mg/ml of SDS in the presence of 2 mM ATP at 20°C for 30 min. The SDS titrated fractions were then loaded on the top of a sucrose (W/W) step gradient constructed with 10 ml of 37.3% (bottom step), 20 ml of 28.8%, and 10 ml of 15% in a Ti 60 tube, and centrifuged at 40,000 rpm for 90 min. The fractions that contain ($Na^+ + K^+$)-ATPase (between 37.3 and 28.8% on the sucrose gradient) were carefully collected and sedimented at 40,000 rpm for 60 minutes. The purified enzyme was resuspended in a sucrose (250 mM)/histidinium chloride (30 mM) buffer, pH 7.2, quick-frozen in liquid nitrogen and stored at −70°C. Highly purified dog kidney ($Na^+ + K^+$)-ATPase was a gift from Dr. Jack Kyte.

Determination of ($Na^+ + K^+$)-ATPase activity. The enzymatic activity was determined as described previously (17) with modifications. Briefly, purified rat or dog ($Na^+ + K^+$)-ATPase was incubated with or without SSA95 or ouabain in the presence of 100 mM Na^+ for 30 min at room temperature. The reaction was initiated by adding 3 mM MgATP and 20 mM K^+ in a final volume of 0.25 ml at 37°C for 30 min and terminated by adding 0.75 ml quench solution and 0.025 ml developer. The color was allowed to develop for 30 min at room temperature and the concentration of phosphate was then determined at 700 nm using a spectrophotometer.

[^{3}H]Ouabain binding. The rat cardiac SL vesicles (2 mg/ml) were incubated with or without purified SSA95 antibody at various concentrations for 30 minutes at room temperature in a medium containing 3 mM MgATP, 100 mM NaCl, and 10 mM Tris/HCl buffer (pH 7.4). [^{3}H]Ouabain (0.13 μM) was then added to the reaction mixture for 30 min at 37°C. Pelleting the samples at 13,000 rpm for 10 min stopped the reaction. The labeled vesicles were washed with 10 mM Tris/HCl buffer and then dissolved in 100 μl 10% SDS solution. The radioactivity of each sample was determined using a β-scintillation counter. The percent of specific [^{3}H]ouabain binding to the enzyme was calculated and compared with control samples in the absence of SSA95.

RESULTS

SSA95 and Its Effect on [^{3}H]Ouabain Binding

To identify potential structural determinants of the ($Na^+ + K^+$)-ATPase that mediate the positive inotropic effect on the heart, we have generated anti-peptide antibodies against the known antigenic sites on the enzyme. Among them, the SSA95 polyclonal antibody

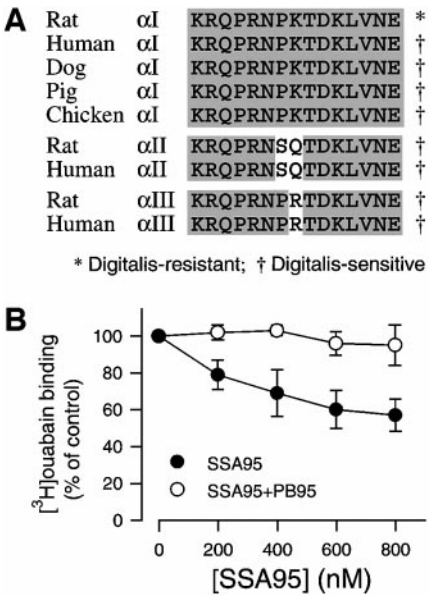

FIG. 1. Site-specific anti-peptide antibody displaces [^{3}H]ouabain binding on the ($Na^+ + K^+$)-ATPase. (A) The amino-acid composition of SSA95 binding region on the ($Na^+ + K^+$)-ATPase. Primary amino-acid sequence of SSA95 binding site is compared among αI type from rat (10), human (12), dog (11), pig (24), chicken (25), and αII and αIII types from rat (26) and human (12) isoforms of ($Na^+ + K^+$)-ATPase. (B) SSA95 displaces [^{3}H]ouabain labeling to cardiac ($Na^+ + K^+$)-ATPase in a dose-dependent manner. Isolated rat cardiac right-side-out SL vesicles were labeled with [^{3}H]ouabain (0.13 μM) in the presence of SSA95. Filled circles: with various concentrations of SSA95 as indicated in the figure. Open circles: in the presence of both SSA95 and PB95 (1:500). The data represent a mean of five experiments.

was raised to specifically recognize the $_{833}$KRQPRNPK-TDKLVNE$_{847}$ peptide (10–12) of the rat α-subunit (18, 19) of the ($Na^+ + K^+$)-ATPase, which is located in the extracellular domain of the enzyme (20–22). Purified antibody recognized both denatured and native ($Na^+ + K^+$)-ATPase of rat cardiac myocytes, as evidenced by Western blots and immunocytostaining, respectively (data not shown). Figure 1A shows that the amino acid sequence of this region is identical among the αI subunits of both digitalis-resistant (e.g., rat (10)) and -sensitive forms (e.g., dog (11) and human (12)) of ($Na^+ + K^+$)-ATPase, and is highly conserved among isoforms of the enzyme as well (12, 23–26).

Cardiac glycosides and related drugs induce positive inotropic effect by binding to the outer cell surface of the α subunit of the ($Na^+ + K^+$)-ATPase (9, 19, 27). To investigate the relationship between the binding sites of SSA95 and cardiac glycosides on the Na^+/K^+-ATPase, [^{3}H]ouabain (0.13 μM) was used to react with isolated rat cardiac sarcolemmal right-side-out vesicles (2 mg/ml) with or without SSA95 at various concentrations. Figure 1B shows that SSA95 antagonized [^{3}H]ouabain binding by reducing the [^{3}H]ouabain labeling by 21, 31, 40, and 43% in the presence of 0.2, 0.4, 0.6, and 0.8 μM SSA95, respectively. However, the

[^{3}H]ouabain labeling was not affected when the immuno-active sites of the SSA95 were saturated by a peptide blocker KRQPRNPKTDKLVNE (PB95) prior to adding [^{3}H]ouabain to the SL vesicles. These results show that SSA95 acts like an antagonist of [^{3}H]-ouabain (Fig. 1B) and suggest that the SSA95 binding region of the enzyme may contain essential structural and functional information for cardiac glycoside binding and the ensuing biological action.

Effect of SSA95 on Cardiac Contraction and Ca^{2+} Transients

We next determined whether the binding of SSA95 to the ($Na^+ + K^+$)-ATPase affects cardiac contractility. Confocal linescan imaging was employed in conjunction with the Ca^{2+}-sensitive indicator, fluo-4, to monitor simultaneously intracellular Ca^{2+} transients and contraction in single rat ventricular myocytes. Cells were continuously excited by electrical stimuli and contracted at 0.5 Hz (Fig. 2). Administration of SSA95 (20 nM, 10–15 min) markedly increased systolic $[Ca^{2+}]_i$ and cell shortenings to 191 ± 24%, ($n = 5$, $P < 0.01$) and 347 ± 51% ($n = 5$, $P < 0.01$) of controls, respectively (Fig. 2A). The diastolic $[Ca^{2+}]_i$ level was also elevated from 100 nM to 161 ± 13 nM ($n = 5$, $P < 0.01$) while accompanying a reduction of the resting cell length (Fig. 2A). Hence, SSA95 exerts a positive inotropic effect in cardiac myocytes via enhancement of diastolic and systolic $[Ca^{2+}]_i$. These results indicate that SSA95 is a novel inotropic antibody and provide the first direct link between the molecular sequence and the contractile action of the ($Na^+ + K^+$)-ATPase in heart cells. To ascertain the specificity of the binding of SSA95 to the ($Na^+ + K^+$)-ATPase, a specific peptide blocker PB95 was mixed with SSA95 prior to administration to cardiac myocytes. Figure 2B shows that this synthetic peptide acted as a SSA95 blocker, and largely abolished the increases both in Ca^{2+} transients and contraction. These results confirmed that the binding of SSA95 to its specific antigenic site is necessary and sufficient for SSA95 to alter intracellular Ca^{2+} handling and to augment contractility in heart cells.

We further quantified the potency and efficacy of SSA95 in modulating cell contractility in rat cardiac myocytes. Figure 2C shows that SSA95 monophasically increased the amplitude of contraction in a dose-dependent manner, with a half effective concentration (EC_{50}) of 11.4 nM. The dose–response curve exhibited a Hill coefficient of 2.55; the threshold and maximal contractile responses were attained at 5 and 50 nM of SSA95, respectively, with the maximum cell contraction increasing four times compared with control (Fig. 2C). These results indicate that SSA95 is a powerful inotropic agent.

Effect of SSA95 on ($Na^+ + K^+$)-ATPase Activity

To investigate whether the binding of SSA95 affects ($Na^+ + K^+$)-ATPase activity, we measured ($Na^+ + K^+$)-ATPase (100 nM) activity in the presence of 100 mM Na^+, 20 mM K^+, 3 mM MgATP, and various concentrations of SSA95 or ouabain. Figure 3 shows that no inhibition of digitalis-resistant rat ($Na^+ + K^+$)-ATPase activity was detected over the concentration range of SSA95 (24–400 nM) where both EC_{50} and the maximum cardiac cell contraction were reached (Figs. 2C and 3A). To exclude the possibility that the inability of SSA95 to inhibit enzymatic activity may be due to the digitalis-resistant nature of rat ($Na^+ + K^+$)-ATPase (Fig. 3), we tested the effect of SSA95 on the digitalis-sensitive ($Na^+ + K^+$)-ATPase (16 nM) purified from dog kidney. Figure 3B shows that ouabain, but not SSA95, inhibited the ATP hydrolysis by up to 47% in the same concentration range (24–400 nM) under the same experimental conditions. Since the SSA95 concentration (400 nM) was 25-times higher than dog ($Na^+ + K^+$)-ATPase (Fig. 3B), this observation further corroborates that the binding of SSA95 to the ($Na^+ + K^+$)-ATPase does not inactivate enzymatic activity. These results indicate that SSA95-enhanced heart cell contraction is independent of inactivation of the ($Na^+ + K^+$)-ATPase.

DISCUSSION

The molecular basis of the ($Na^+ + K^+$)-ATPase that regulates excitation/contraction of the heart is not completely understood. In the present study, we have identified a structural region of the ($Na^+ + K^+$)-ATPase that directly participates in the regulation of cardiac contractility. This was demonstrated by the binding of a site-specific antibody (SSA95) to the ($Na^+ + K^+$)-ATPase, resulting in a marked increase of the Ca^{2+} transients and contraction in rat cardiac mocytes (Fig. 2A). Moreover, experimental data show that SSA95 increased heart cell contractile force in a dose-dependent fashion with an EC_{50} of 11.4 nM (Fig. 2C), demonstrating that SSA95 induces a positive inotropic action and that SSA95 is a potent inotropic reagent. The SSA95-induced biological effects on heart cells were completely abolished by a peptide blocker PB95 (Fig. 2B), further indicating the specificity of the SSA95 action.

To date, there are two major conflicting molecular models regarding the localization of the KRQPRNPKTDKLVNE epitope. Model I suggests that this epitope is located on the extracellular side of membrane (20–22). Model II suggests that it is in the intracellular side (28). Our data are consistent with the Model I topology of the ($Na^+ + K^+$)-ATPase regarding this epitope, since SSA95 is not membrane permeable and binds tightly to the membrane surface of the ($Na^+ + K^+$)-ATPase to induce a marked biological effect. Shull *et al.* (20) first

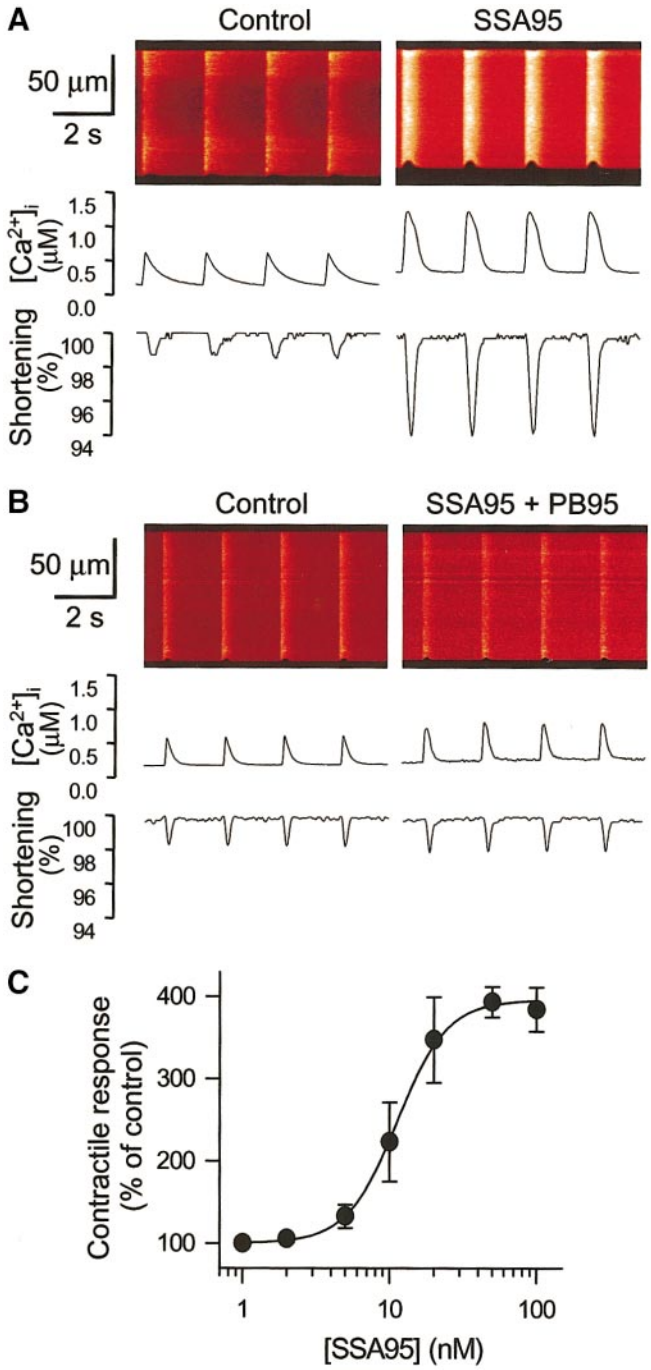

FIG. 2. Effects of SSA95 on cell contraction and intracellular Ca^{2+} transients in rat heart cells. (A) Simultaneous recordings of confocal linescan images (top), intracellular Ca^{2+} transients (middle), and cell shortening (bottom) in the absence (control) and presence of SSA95 (20 nM for 15 min). Note that SSA95 markedly increases cell contraction amplitude under a constant beating rate, accompanying an elevation of both diastolic and systolic $[Ca^{2+}]_i$. (B) The same as in A, except that SSA95 was mixed with the specific peptide blocker (PB95, 20 μM) prior to administration to the cell. The peptide blocker largely impeded the SSA95 effects on $[Ca^{2+}]_i$ and contraction. (C) Dose-dependent contractile response of SSA95 in rat ventricular myocytes. The sigmoid dose–response curve of SSA95 fits the equation $R = 1 + (R_{max} - 1)/[1 + (EC_{50}/C)^h]$, where R is the contractile response expressed in percentage of control, R_{max} is the maximal contractile response, C is the SSA95 concentration, EC_{50} is the C at the half maximal contractile response, and h is the Hill coefficient. Curve fitting yielded the parameters R_{max} = 396%, h = 2.55 and EC_{50} = 11.4 nM. n = 4–6 for each data point.

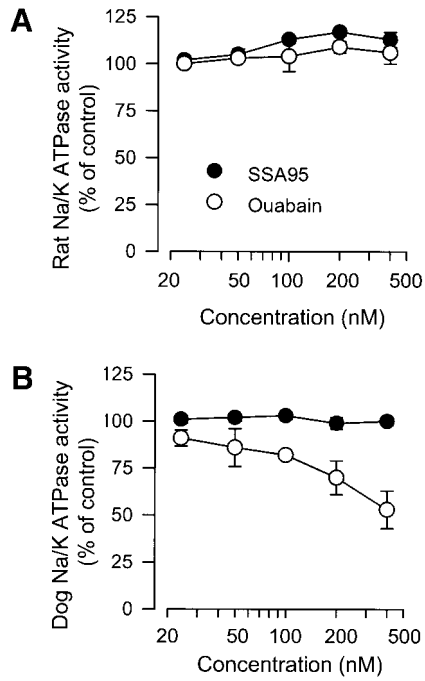

FIG. 3. Effect of SSA95 and ouabain on $(Na^+ + K^+)$-ATPase activity. (A) Digitalis-resistant rat $(Na^+ + K^+)$-ATPase (100 nM). (B) Digitalis-sensitive dog Na^+/K^+-ATPase (16 nM). Filled circles: SSA95. Open circles: ouabain. Note that ouabain at the concentrations used did not inactivate ouabain-resistant rat $(Na^+ + K^+)$-ATPase, but reduced the enzymatic activity of the ouabain-sensitive dog $(Na^+ + K^+)$-ATPase. Note that SSA95 inactivated neither regardless of cardiac glycoside sensitivity. The data represent a mean of four experiments.

predicted the primary amino acid composition of the H5-H6 domain (Model I) of the α-subunit of $(Na^+ + K^+)$-ATPase based on the hydropathy values of membrane-spanning segments of the enzyme. Ovchinnikov *et al.* (22) confirmed the H5–H6 domain (Model I) sequence and further demonstrated its extracellular localization. The $_{833}$KRQPRNPKTDKLVNE$_{847}$ region (rat numbering), as a unique antigenic binding site of SSA95, is a part of the H5-H6 domain (Model I) of the rat Na^+/K^+-ATPase. SSA95 acts from the outside of intact myocytes to increase intracellular Ca^{2+} concentration and induce a positive inotropic action, supporting the concept that the KRQPRNPKTDKLVNE epitope of the $(Na^+ + K^+)$-ATPase is located at the extracellular side of the enzyme (20–22) Furthermore, preliminary results from the *in vivo* mouse heart study showed that SSA95 induced a positive inotropic action on mouse heart as demonstrated in terms of intraventricular pressure-volume loops after administration of SSA95 (unpublished data). The results of this *in vivo*

study further suggest that the binding site of the inotropic antibody is accessible from the membrane surface. Whether this region of the $(Na^+ + K^+)$-ATPase might dynamically flip in and out of the membrane during the transitions among four conformations of the enzyme while Na^+ and K^+ cations traverse the plasma membrane, remains to be determined. To examine the possibility that SSA95 might bind to Ca^{2+}-ATPase to induce an inotropy, Western blotting was performed using the rat heart homogenate. The results show that SSA95 specifically detected $(Na^+ + K^+)$-ATPase in both rat heart homogenates (Fig. 4A, lane 2) and dog kidney vesicles (Fig. 4A, lane 3), but recognized neither sarcolemma Ca^{2+}-ATPase (Fig. 4A, lane 2), nor sarcoplasmic reticulum Ca^{2+}-ATPase (Fig. 4B). These results further substantiate that SSA95 specifically binds to the $(Na^+ + K^+)$-ATPase to induce its physiological action.

According to the reported mechanisms of digitalis-induced inotropic action, digitalis inhibits the $(Na^+ + K^+)$-ATPase which, in turn, increases the intracellular Na^+ ion concentration $[Na^+]_i$. The increase in $[Na^+]_i$ leads to a rise in intracellular calcium $[Ca^{2+}]_i$ level by affecting Na^+/Ca^{2+} exchange. The increase in the $[Ca^{2+}]_i$ level results in a positive inotropic effect. Therefore, the positive inotropic effect is secondary to an increase in $[Na^+]_i$ after inhibition of the $(Na^+ + K^+)$-ATPase. However, SSA95 did not inhibit the $(Na^+ + K^+)$-ATPase activity (Fig. 3) while increasing the force of contraction of the heart cells (Fig. 2A), indicating that the SSA95-induced positive inotropic effect is not coupled with the inactivation of the enzyme under our experimental conditions. Whether the binding of SSA95 alters $[Na^+]_i$, we tested the effect of SSA95 on $[Na^+]_i$ in CV-1 African Green monkey cells (American Type Culture Collection). Our pilot study shows that the $[Na^+]_i$ was increased following the binding of SSA95 (unpublished data), suggesting that the mechanistic pathway underlying SSA95-induced excitation/contraction involves the changes of $[Na^+]_i$. Both SSA95 and digitalis glycosides bind to the $(Na^+ + K^+)$-ATPase on the membrane surface and affect $[Na^+]_i$ and $[Ca^{2+}]_i$ to increase the force of contraction. Whether they share a similar mechanistic pathway to alter $[Na^+]_i$ and $[Ca^{2+}]_i$ through a Na^+/Ca^{2+} exchanger (29) remains to be determined. Nevertheless, the discovery that SSA95, as a potent non-inactivating inotropic reagent, may provide potential new therapeutic strategies to improve the function of the failing heart in cardiovascular medicine.

SSA95 antagonizes the ouabain binding by reducing the [^{3}H]ouabain labeling to the $(Na^+ + K^+)$-ATPase (Fig. 1B). The specific peptide blocker PB95 eliminated this antagonist effect of SSA95 (Fig. 1B) further emphasizing the specificity of SSA95 in competitive [^{3}H]ouabain labeling. These results imply that the antigenic site of SSA95 may contain one of the digitalis glycoside interacting-sites,[30] located in the drug-binding pocket of the enzyme. We hypothesize that it is the interaction of SSA95 or digitalis glycosides to this biologically active region of the $(Na^+ + K^+)$-ATPase that leads to the positive inotropic action. However, we are not ruling out the possibility that the binding of SSA95 to the $(Na^+ + K^+)$-ATPase may cause a conformational change (2, 21) of the enzyme, and this in turn may influence the binding of digitalis glycosides to the $(Na^+ + K^+)$-ATPase. The observations that SSA95 antagonized [^{3}H]ouabain binding (Fig. 1) and increased cardiac myocytes contraction without inactivating

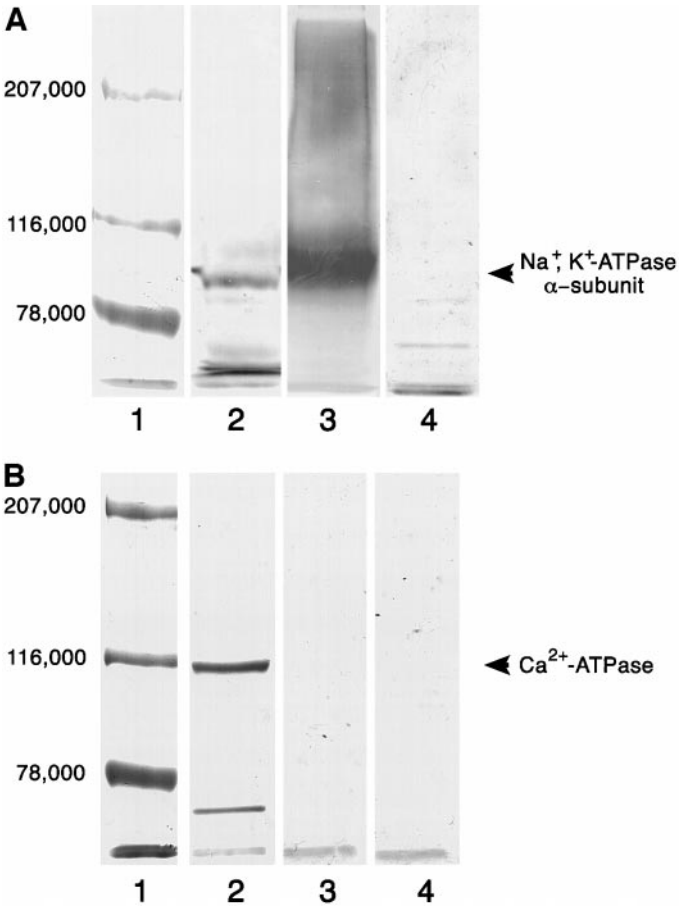

FIG. 4. Specific binding of SSA95 to the α-polypeptide of $(Na^+ + K^+)$-ATPase in Western transfer analysis. Samples were dissolved by the Laemmli sample buffer (Bio-Rad) and separated by electrophoresis on a gel of 5% polyacrylamide. The separated polypeptides were then transferred to nitrocellulose membranes and reacted with SSA95 directed against the sequence –KRQPRNPKTDKLVNE followed by goat anti-rabbit IgG (Fc) to which alkaline phosphatase (AP) was attached. The distribution of bound antibody was determined by AP-dependent staining. (A) Binding of SSA95 to $(Na^+ + K^+)$-ATPase. (B) Binding of SSA95 to rat cardiac sarcoplasmic reticulum (SR) vesicles. Lanes A1 and B1: Standard polypeptides. Lane A2: Rat heart homogenate (30 μg) with SSA95. Lane A3: Dog SL vesicles (20 μg) with SSA95. Lane A4: Rat heart homogenate with secondary antibody only. Lane B2: SR vesicles (20 μg) with SERCA2 (anti-SR Ca^{2+}-ATPase). Lane B3: SR vesicles with SSA95. Lane B4: SR vesicles with secondary antibody only. SSA95 specifically binds to the $(Na^+ + K^+)$-ATPase in rat heart homogenates and dog SL vesicles, but not to both SL and SR Ca^{2+}-ATPases.

$(Na^+ + K^+)$-ATPase (Fig. 3) support the notion[31] that the inhibition of the $(Na^+ + K^+)$-ATPase may not be the sole factor to generate a positive inotropic action.

The present study reports that a site-specific antibody to the $(Na^+ + K^+)$-ATPase exerts a potent biological effect in cardiac myocytes and demonstrates a key structural region of the enzyme that participates in the regulation of cardiac contractility. These results establish an important link between a biological action and a precise molecular structure of the $(Na^+ + K^+)$-ATPase. Furthermore, the observation that the SSA95-induced positive inotropic effect is independent of inactivation of the enzyme may reveal a novel mode for $(Na^+ + K^+)$-ATPase to regulate cardiac function. Our experimental data provide new molecular insights into the structural and functional relationship of the ubiquitous $(Na^+ + K^+)$-ATPase.

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