Alterations in the Plasma Membrane Properties of the Myocardium of Spontaneously Hypertensive Rats

RAM V. SHARMA, CAROL A. BUTTERS, AND RAMESH C. BHALLA

SUMMARY Spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) rats were used to investigate the adaptive biochemical changes in the myocardium in response to chronic afterload. Ouabain-inhibited Na⁺, K⁺-adenosine triphosphatase (ATPase) activity was decreased by 40% in myocardium of SHR compared with that from WKY, which may lead to increased intracellular Ca²⁺ through Na⁺-Ca²⁺ exchange. Similarly, α₁-adrenergic receptor density, estimated by [³H]prazosin binding, was decreased by 42% in myocardial membranes of SHR, while the affinity for the agonist and the antagonist was not altered. In contrast, the number of Ca²⁺ channels estimated by [³H]nitrendipine binding was increased by 45% in myocardial membranes of SHR, while the affinity was comparable between WKY and SHR. These differences between WKY and SHR in the membrane properties were not due to differential contamination of plasma membranes because the activities of other putative plasma membrane marker enzymes were comparable between WKY and SHR. There were no differences between WKY and SHR in the myosin ATPase activity estimated using myofibrils, actomyosin, and myosin. These results suggest that specific alterations have occurred in the plasma membrane properties of myocardium of SHR that result in altered intracellular Ca²⁺ metabolism. These alterations may have an important bearing on excitation-contraction coupling in myocardium of SHR. (Hypertension 8: 583-591, 1986)

KEYWORDS • spontaneously hypertensive rats • plasma membranes • Na⁺, K⁺-ATPase • α₁-adrenergic receptors • Ca²⁺ channels • myosin ATPase
One of the most prominent actions of catecholamines on the heart is their ability to increase the influx of Ca$^{2+}$ into myocardial cells. The positive inotropic and chronotropic responses to catecholamines usually have been assumed to be due to the activation of $\beta$-adrenergic receptors. However, phenylephrine and other $\alpha$-adrenergic receptor agonists have been shown to cause positive inotropy in the heart, an action that can be blocked by phentolamine. It is, therefore, reasonable to assume that the $\alpha$-adrenergic receptor mediated mechanism in hypertrophied hearts from SHR may be considerably different from that occurring in control hearts. Thus, it was logical to investigate whether changes in the number and affinity of $\alpha$-adrenergic receptors have occurred in the myocardium of SHR.

In certain models of cardiac hypertrophy the reduced cardiac function is associated with a decrease in contractile protein ATPase activity, and this decrease has been attributed to a shift in myosin isoenzyme from $V_1$ to $V_2$. These changes have important functional implications because the shortening velocity of cardiac muscle and cross-bridge cycling have been shown to be correlated with myosin ATPase activity and myosin isoenzyme composition. To understand the adaptive biochemical changes in response to chronic afterload, we have investigated plasma membrane properties with respect to Na$^+$,K$^+$-ATPase activity, the number of Ca$^{2+}$ channels and $\alpha$-adrenergic receptors and contractile protein ATPase activity in young adult SHR with moderate left ventricular hypertrophy.

Materials and Methods

Age-matched (16-week-old), male SHR and WKY were used (70 and 80 animals, respectively; Taconic Farms, Germantown, NY, USA). All rats were weighed biweekly, and blood pressure was measured by the tail-cuff method in conscious rats. The rats were maintained under identical conditions and given Purina rodent laboratory chow (St. Louis, MO, USA) and tap water ad libitum.

**Drugs**

The $[^{3}H]$prazosin (12.3 Ci/mmol) was purchased from Amersham (Arlington Heights, IL, USA), and $[^{3}H]$nitrendipine (79 Ci/mmol) was purchased from New England Nuclear (Boston, MA, USA). Unlabeled prazosin and nifedipine were generous gifts from Burroughs-Wellcome (Research Triangle Park, NC, USA). $l$-Norepinephrine bitartrate was purchased from Sigma Chemical (St. Louis, MO, USA). Nifedipine stock solution was made in absolute alcohol. Prazosin stock solution was made in 0.01 M HCl in 50% ethanol. The $l$-norepinephrine stock solution was made in buffer. All solutions were made fresh daily just before starting the experiment. Concentration of ethanol in the assay was less than 0.5% and did not affect $[^{3}H]$nitrendipine binding. All other chemicals and biochemicals were of analytical grade and purchased from local vendors.

**Isolation of Myofibrils, Actomyosin, Myosin, and Actin**

The rats were killed by direct heart puncture while under ether anesthesia, and their hearts were removed. The coronary circulation then was flushed through the aorta with physiological salt solution and the ventricles were excised, blotted on filter paper, and weighed to calculate ventricle weight/body weight ratio as an index of cardiac hypertrophy. The right ventricle then was removed, and the left ventricle along with septum was ground in liquid nitrogen and stored in individual vials at $-100^\circ C$ for estimation of contractile protein ATPase activity. Myofibrils, free of mitochondria, sarclemma, and sarcoplasmic reticulum, were prepared from individual left ventricles, essentially by the method of Solaro et al. The sodium dodecyl sulfate polyacrylamide gels of myofibrils for different groups did not show any differences in the protein profiles. Actomyosin was extracted from the myofibrils. Myosin was prepared from left ventricles pooled from two to three animals by the method of Ofer et al. Purity of myosin was checked by sodium dodecyl sulfate gel electrophoresis. Myosin was free of contamination from actin, troponin, and tropomyosin in all preparations. The 280:260 ratio was between 1.45 and 1.55 for all batches of myosin prepared. Myosin was dialyzed against 0.5 M KCl, 0.1 mM ethylenediaminetetraacetic acid (EDTA), 0.1 mM dithiothreitol, 1 mM NaN$_3$, and 10 mM tris(hydroxyethyl)aminomethane (Tris) HCl, pH 7.6, for 36 hours before use. Actin was purified from rabbit skeletal muscle acetone powder by the method of Spudick and Watt.

**ATPase Assay**

All contractile protein ATPase activities were estimated at 30°C. Myofibrillar ATPase activity was determined in 40 mM imidazole HCl (pH 7.0), 3.16 mM Mg$^{2+}$ adenosine 5'-triphosphate (ATP), 0.316 mM free Mg$^{2+}$, 5.5 mM NaN$_3$, and 57 mM KCl. Myofibrillar protein concentration was 400 $\mu$g/ml. The assay medium contained 4 mM ethylene glycol bis(β-aminoethyl)-N,N',N'-tetraacetic acid (EGTA) and the appropriate concentration of CaCl$_2$ to give 1 $\mu$M free Ca$^{2+}$. The apparent binding constant for Ca$^{2+}$-EGTA used for the calculation of free Ca$^{2+}$ concentration was 10$^{-6}$ M. Actomyosin ATPase activity was measured in 50 mM Tris HCl (pH 7.6), 5 mM ATP, 5 mM NaN$_3$, 10 mM CaCl$_2$, and 500 mM KCl. Actomyosin concentration was 200 $\mu$g/tube. Myosin ATPase activity was determined in 1) K$^+$-EDTA-activated reaction mixture (50 mM Tris HCl [pH 7.6], 5 mM EDTA, 500 mM KCl, 5 mM Na$_3$P$_2$O$_7$, 5 mM ATP, 100 $\mu$g protein) and 2) Ca$^{2+}$-activated reaction mixture (50 mM Tris HCl [pH 7.6], 500 mM KCl, 5 mM NaN$_3$, 5 mM ATP, 10 mM CaCl$_2$, 100 $\mu$g myosin). Actin-activated myosin ATPase activity was assayed in 50 mM imidazole HCl (pH 7.0), 0.1 mM dithiothreitol, 1.5 mM ATP, 2.0 mM MgCl$_2$, 1 mM NaN$_3$, 50 mM KCl, and 26 $\mu$M skeletal muscle actin. Reactions were started by adding ATP, and reaction volume was 1 ml in all ATPase assays. The inorganic phosphate (P) liberation was measured by the method of Fiske and Subbarow.

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Protein concentration was determined by the method of Bradford\textsuperscript{24} using bovine serum albumin as the standard.

**Preparation of Plasma Membranes**

Cardiac plasma membranes were prepared identically for each group of rats. All steps were performed at 4°C. Rats were killed by direct heart puncture while under ether anesthesia, and atria, connective tissue, and major vessels were removed. Individual hearts were weighed, and 10 to 15 hearts were then combined for each preparation. The tissue was homogenized in 10 volumes of homogenizing buffer (approximately 140–160 ml) containing 20 mM Tris maleate (pH 6.8), 0.25 M sucrose, 1 mM dithiothreitol, and 0.1 mM phenylmethylsulfonyl fluoride with a Polytron homogenizer (Model PT 10; Brinkmann Instruments, Westbury, NY, USA) at half-maximum speed for three 10-second bursts alternated with 1-minute rest intervals. The resulting homogenate was centrifuged at 2000 rpm for 10 minutes in a Sorvall SS-34 rotor (Norwalk, CT, USA), and the supernatant was then centrifuged at 8500 rpm for 20 minutes in a Sorvall SS-34 rotor. The supernatant from this step was centrifuged at 30,000 rpm for 30 minutes in a Beckman 35 rotor. The resulting pellet was designated as microsomes, or 200 to 250 mg tissue was layered over a 5-ml cushion of 45% sucrose in 37% sucrose (3 mg/ml), and 7 ml of this suspension containing 20 mM Tris maleate (pH 6.8), 1 mM dithiothreitol, and 0.1 mM phenylmethylsulfonyl fluoride. The extracted microsomes were suspended in 37% sucrose (3 mg/ml), and 7 ml of this suspension was layered over a 5% (wt/vol) cushion of 45% sucrose in each gradient tube. The gradients were centrifuged overnight at 27,000 rpm using a Beckman SW-27 rotor. The accumulated membranes at the interface of the 10 to 28% sucrose were collected, the concentration of 1 mg/ml. Membranes were assayed for enzyme activity either immediately or following storage at −70°C for up to 3 weeks.

**5’-Nucleotidase**

5’-Nucleotidase was measured in a total assay volume of 1000 µl consisting of a 20-µl protein sample and 980 µl of enzyme substrate. The reaction mixture contained 5 mM adenosine 5’-monophosphate (AMP), 10 mM MgCl\textsubscript{2}, and 100 mM Tris HCl (pH 7.6). The protein concentration used in the assays was 4 to 5 µg plasma membrane fraction, 20 to 30 µg microsomes, or 50 to 60 µg homogenate. The incubation time was 5 minutes at 37°C. The reaction was terminated by adding 5 ml of malachite green reagent, and the P, was determined.\textsuperscript{25} Protein sample blanks were necessary for these assays because the homogenate gives color with malachite green. This color is probably due to the large quantities of phosphate liberated from the tissue high-energy phosphates during hydrolysis with the 1 N HCl present in the malachite green reagent.

**Phosphodiesterase I**

Phosphodiesterase I was measured in a total volume of 600 µl. The reaction mixture consisted of 1 mM thymidine 5’-monophosphate-p-nitrophenyl ester (Sigma) in 20 mM Tris. The protein used in the assays was 10 to 15 µg plasma membrane fraction, 50 to 60 µg microsomes, or 200 to 250 µg postnuclear supernatant. Enzyme activity was measured at 37°C for 30 minutes. The reaction was terminated by adding 1.5 ml of 20 mM NaOH. The nitrophenol released was measured spectrophotometrically at 400 nm.

**Na\textsuperscript{+},K\textsuperscript{+}-ATPase**

The Na\textsuperscript{+},K\textsuperscript{+}-ATPase was measured in a total volume of 500 µl. The reaction mixture contained 30 mM imidazole HCl (pH 7.0), 120 mM NaCl, 10 mM KCl, 3 mM MgCl\textsubscript{2}, 3 mM Na\textsubscript{2}ATP, 0.5 mM EGTA, and 5 mM NaN\textsubscript{3} with or without 1 mM ouabain. The Mg\textsuperscript{2+}-ATPase activity was determined under the same conditions except that NaCl, KCl, and ouabain were omitted. The proteins used in this assay were 4 to 5 µg plasma membrane fraction, 20 to 30 µg microsomes, or 50 to 60 µg homogenate. Enzyme activity was measured at 37°C for 1 to 10 minutes. Reactions were stopped by adding 1 ml ice-cold reagent containing 10% (wt/vol) trichloroacetic acid instead of HCl and immediately placing the tubes in ice. The inorganic phosphate production was measured according to the method of Ottolenghi.\textsuperscript{26}

**Binding of [\textsuperscript{3}H]Nitrendipine to Plasma Membranes**

Binding of [\textsuperscript{3}H]Nitrendipine was measured in a total volume of 1 ml containing 0.03 to 1 nM [\textsuperscript{3}H]Nitrendipine, 50 mM Tris HCl (pH 7.4), and 100 µM Ca\textsuperscript{2+} at 30°C. After a 60-minute incubation, samples were filtered through Whatman GF/C glass fiber filters (Clifton, NJ, USA) under vacuum. The filters were washed three times with 5 ml of ice-cold 50 mM Tris HCl (pH 7.4) containing 100 µM Ca\textsuperscript{2+} and placed in scintillation vials. The [\textsuperscript{3}H]nitrendipine binding in the presence of 10\textsuperscript{-7} M nonlabeled nifedipine was subtracted to obtain specific binding. The [\textsuperscript{3}H]nitrendipine binding experiments were performed under a sodium lamp to prevent deterioration of these compounds due to light.

**Binding of [\textsuperscript{3}H]Prazosin to Plasma Membranes**

Binding of [\textsuperscript{3}H]prazosin was measured during a 60-minute incubation at 30°C in a total volume of 1 ml 50 mM Tris HCl (pH 7.4) and 10 mM MgCl\textsubscript{2}. Reactions were initiated by adding 20 µl of purified plasma membrane fraction and incubated at 37°C for 5 minutes. The reaction was terminated by adding 5 ml of malachite green reagent, and the P, was determined.
membranes (25–30 μg membrane protein) to an assay mixture containing [3H]prazosin and unlabeled prazosin. The assay mixture included [3H]prazosin at a final concentration of 0.04 to 2.8 nM with or without 0.1 μM unlabeled prazosin to determine nonspecific binding. Dissociation constant ($K_d$) and maximum binding ($B_{max}$) values were calculated by Scatchard analysis. In the experiments measuring the displacement of [3H]prazosin by α-adrenergic ligands, the assay conditions were changed to provide a larger volume. In these experiments the total assay volume was 5 ml, containing 50 mM Tris HCl (pH 7.4), 10 mM MgCl$_2$, and 0.15 nM [3H]prazosin. Binding was terminated in all experiments by the addition of 5 ml of ice-cold 50 mM Tris HCl (pH 7.4) and 10 mM MgCl$_2$, followed by rapid filtration through Whatman GF/C filters. The filters were then washed three times with 5 ml of wash buffer, dried at 60°C for 1 hour, and counted in 10 ml of Beckman HP scintillant. All results are expressed as means of triplicate determinations after subtracting nonspecific binding in the presence of 0.1 μM unlabeled prazosin.

Results

Table 1 shows the blood pressure, body weight, and heart weight/body weight ratio of SHR and WKY. The body weight of SHR was significantly less than that of WKY, while their heart weight/body weight ratio and blood pressure were significantly greater.

The protein yield of plasma membrane vesicles (mg/g, ventricular weight/tissue weight) was comparable between WKY and SHR. The putative marker enzyme activities of 5'-nucleotidase, phosphodiesterase I, alkaline phosphatase, and ouabain-inhibited Na$^+$.K$^+$.ATPase were enriched 30-fold to 35-fold as compared with homogenates in both WKY and SHR (Figure 1). Furthermore, no differences in the specific activity of 5'-nucleotidase, phosphodiesterase I, and alkaline phosphatase were observed between WKY and SHR.

The ouabain-sensitive Na$^+$.K$^+$.ATPase activity was determined in homogenate as well as plasma membrane preparations of myocardium from SHR and WKY. In both homogenate and plasma membranes, the Na$^+$.K$^+$.ATPase activity was significantly decreased in hearts of SHR as compared with those of WKY (see Figure 1). The enzyme activity was reduced by 36% in the homogenate and 43% in the plasma membranes of myocardium from SHR as compared with that in WKY.

The number of α$_1$-adrenergic receptors in the myocardial membranes of WKY and SHR was estimated by using different concentrations of [3H]prazosin. The specific binding was higher in the plasma membranes of WKY than in those of SHR at all concentrations of [3H]prazosin. The $B_{max}$ value for SHR was 45% lower than that for WKY, with no change in the $K_d$ value calculated by Scatchard analysis (Figure 2 and Table 2). Similarly, the ligand affinity was also estimated by studying the inhibition of [3H]prazosin using different concentrations of unlabeled prazosin and norepinephrine (Figure 3). The inhibition constant ($K_i$) values for inhibition of [3H]prazosin-specific binding are given in Table 2; there were no differences between WKY and SHR in $K_i$ values for prazosin and norepinephrine. These results indicate that the receptor density is decreased in SHR as compared with that in WKY, whereas α$_1$-adrenergic receptor affinity is not altered in the myocardium of SHR.

[3H]Nitrendipine binding was studied using homogenate and purified plasma membranes of myocardium from WKY and SHR. Because nitrendipine binding to

![Figure 1. Plasma membrane (PM) marker enzyme activities in cardiac homogenates and plasma membranes from WKY and SHR isolated by discontinuous sucrose density gradient centrifugation of microsomes extracted with 0.6 M KCl to remove contractile proteins. All assays were done under optimal conditions at 37°C as described in Methods, and the substrate hydrolysis was kept under 20%. Results are mean ± SEM for three separate experiments performed on different batches of plasma membrane preparations. Asterisk indicates significant ($p<0.05$) difference between SHR and WKY. Pi = inorganic phosphate.]

Table 1. Body Weight, Heart Weight to Body Weight Ratio, and Blood Pressure of 80 SHR and 80 WKY

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WKY</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>352 ± 9</td>
<td>276 ± 5.0*</td>
</tr>
<tr>
<td>Heart/body weight (mg/g)</td>
<td>3.0 ± 0.05</td>
<td>3.7 ± 0.1*</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>139 ± 3.0</td>
<td>212 ± 4.0*</td>
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</tbody>
</table>

Values are means ± SEM.

* $p < 0.05$, compared with values in WKY.
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**Figure 2.** Scatchard analysis of [3H]prazosin binding to cardiac plasma membranes from SHR and WKY. Binding was done at 30°C for 1 hour in a total volume of 1 ml 50 mM Tris HCl (pH 7.4) and 10 mM MgCl2, containing approximately 30 µg of protein and 0.04 to 2.8 nM [3H]prazosin. Nonspecific binding observed in the presence of 10⁻⁷ M unlabeled prazosin was subtracted. Data are representative of three separate experiments. B/F = bound/free.

**Table 2.** [3H]Prazosin Binding Constants and Inhibition Constant Values for [3H]Prazosin Displacement by Unlabeled Ligands

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WKY</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binding constants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( K_d ) (nM)</td>
<td>0.126 ± 0.0078</td>
<td>0.129 ± 0.015</td>
</tr>
<tr>
<td>( B_{max} ) (fmol/mg)</td>
<td>1120 ± 13.5</td>
<td>611 ± 28.4*</td>
</tr>
<tr>
<td>( K_i ) values (nM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prazosin</td>
<td>0.082 ± 0.01</td>
<td>0.087 ± 0.015</td>
</tr>
<tr>
<td>L-Norepinephrine</td>
<td>2277 ± 380</td>
<td>2100 ± 349</td>
</tr>
</tbody>
</table>

Values are means ± SEM of three experiments.

\( K_d \) and \( B_{max} \) values were calculated by Scatchard analysis (see Figure 2). The 50% inhibitory concentration (IC₅₀) values were determined graphically (see Figure 3). The \( K_i \) values were calculated from the equation \( K_i = IC_50/(1 + S/K_d) \), where \( S \) is the concentration of [3H]prazosin (150 pM) used in the assay, and \( K_d \) is the dissociation constant calculated by Scatchard analysis (126 pM).

*p < 0.05, compared with values in WKY.

**Figure 3.** Displacement of specific [3H]prazosin binding to rat cardiac plasma membranes of SHR (●) and WKY (○) with prazosin and norepinephrine. Binding was performed at 30°C for 1 hour in 5 ml 50 mM Tris HCl (pH 7.4) and 10 mM MgCl₂, containing approximately 30 µg of membrane protein and 0.15 nM [3H]prazosin together with indicated final concentrations of prazosin and norepinephrine. Results are mean ± SEM of three separate experiments.

the cardiac membranes has been shown to saturate between 0.5 and 1 nM,4 in some of these experiments the nitrendipine binding sites were estimated using 0.03 to 1 nM [3H]nitrendipine. The nonspecific binding (binding in the presence of 0.1 µM unlabeled nifedipine) was less than 25% of the total binding in all experiments. Nonspecific binding was subtracted to calculate specific binding. Scatchard analysis of specific [3H]nitrendipine binding revealed a single class of high affinity binding sites (Figure 4). The \( K_d \) values were comparable for WKY (0.271 nM) and SHR (0.279 nM); however, the number of [3H]nitrendipine binding sites was significantly increased (\( p < 0.05 \)) in both homogenate and plasma membranes of myocardium of SHR as compared with that in WKY (Figure 5).

The protein yield of myofibrils, actomyosin, and myosin was comparable in SHR and WKY. Myofibrillar ATPase activity was determined at 10⁻⁶ M free Ca²⁺, which was found to be suitable for maximum activation of myofibrillar Ca²⁺-activated Mg²⁺-ATPase activity. Myofibrillar ATPase activity was not significantly different between WKY and SHR, nor was actin-activated myosin ATPase activity of purified...
myosin (Figure 6). Similarly, Ca\textsuperscript{2+}-stimulated acto-
myosin and myosin ATPase activities were not differ-
ent between groups when assayed in the presence of 10
mM Ca\textsuperscript{2+} and 500 mM K\textsuperscript{+} (see Figure 6).

Discussion

The findings of this study reveal the following sig-
nificant alterations in the myocardial membrane prop-
erties of SHR as compared with those of WKY: 1) oua-
bain-sensitive Na\textsuperscript{+},K\textsuperscript{+}-ATPase activity was de-
creased by 40\%, 2) \alpha\textsubscript{1}-adrenergic receptor density was de-
creased by 43\%, and 3) Ca\textsuperscript{2+} channel density was in-
creased by 42\%. On the other hand, the myosin
ATPase activity of myofibrils, actomyosin, and puri-
fied myosin were comparable in WKY and SHR,
which suggests that moderate compensatory cardiac
hypertrophy is not associated with changes in myosin
ATPase activity. These results are in agreement with
the observations of Lauva and Sen,\textsuperscript{27} who found no
differences in the myosin isoenzyme distribution be-
tween WKY and SHR up to 24 weeks of age. How-
ever, studies in older rats (36–52 weeks of age)
showed differences in the myosin isoenzyme pattern\textsuperscript{28,29} and a decrease in myofibrillar ATPase activ-
ity.\textsuperscript{30} These data suggest that changes in myosin isoen-
zyme distribution and myosin ATPase activity in SHR
appear after 8 to 9 months of age.

Our results also demonstrated that the ouabain-sen-
sitive Na\textsuperscript{+},K\textsuperscript{+}-ATPase activity was decreased by 35 to
45\% in both homogenates and purified plasma mem-
branes of myocardium from SHR. The decrease in the
Na\textsuperscript{+},K\textsuperscript{+}-ATPase activity in the plasma membranes of
SHR myocardium does not appear to be due to differ-
ential contamination of sarcolemma with contractile
proteins or other subcellular organelles for the follow-
ing reasons: 1) the protein yield of plasma membranes
(mg/g wet tissue weight) was comparable in WKY and
SHR; 2) the activity of other putative plasma mem-
brane marker enzymes (i.e., 5'-nucleotidase, phos-
phodiesterase I, and alkaline phosphatase) was compa-
rable in both groups of membrane preparations; 3)
[\textsuperscript{3}H]nitrendipine binding sites were increased 30-fold
to 35-fold in myocardial membranes from SHR as
compared with those from WKY. Thus, the decrease
in Na\textsuperscript{+},K\textsuperscript{+}-ATPase activity in myocardium from SHR
appears to be specific. Our observations of decreased
Na\textsuperscript{+},K\textsuperscript{+}-ATPase activity in the myocardium from
SHR are consistent with earlier observations.\textsuperscript{12,31}

The effect of reduced Na\textsuperscript{+},K\textsuperscript{+}-ATPase activity on
myocardial function of SHR is not clearly understood.
Several studies have suggested that the inhibition of
Na\textsuperscript{+},K\textsuperscript{+}-ATPase activity results in elevated intracellu-
lar Na\textsuperscript{+} levels, which in turn lead to increased intracel-
ular Ca\textsuperscript{2+} concentrations due to either increased Ca\textsuperscript{2+}
 influx\textsuperscript{8,9,32,33} or decreased Ca\textsuperscript{2+} efflux through Na\textsuperscript{+}–
Ca\textsuperscript{2+} exchange.\textsuperscript{7,33,34} It is therefore possible that the
reduced Na\textsuperscript{+},K\textsuperscript{+}-ATPase activity and the increased
number of Ca\textsuperscript{2+} channels in the myocardium of SHR
observed in these studies could augment the myocardi-
al contractility due to increased availability of Ca\textsuperscript{2+}
through the mechanisms just discussed. These changes may represent compensatory mechanisms for left ventricular function and myocardial mechanics in young adult SHR to cope with the elevated peripheral resistance.

The number and affinity of $[^3H]$nitrendipine binding sites observed in this study in plasma membrane from WKY are comparable to those reported for rat and dog heart subcellular fractions.\textsuperscript{4,6} We also observed a significant increase in the number of $[^3H]$nitrendipine binding sites in myocardium from SHR as compared with that from WKY with no differences in its affinity. On the other hand, an earlier study found only a slight increase of membrane phosphatidylinositides and the release of inositol bis-phosphates and tris-phosphates.\textsuperscript{41-43} Recently, addition of IP$_3$ to the cardiac sarcoplasmic reticulum, or do both (for review see Reference 14). Considerable evidence has accumulated from various types of mammalian cells that stimulation of $\alpha_1$-adrenergic receptors mediates intracellular Ca$^{2+}$ release through the breakdown of membrane phosphatidylinositides and the release of inositol bis-phosphates and tris-phosphates.\textsuperscript{41-43} Recently, IP$_3$ has been shown to release Ca$^{2+}$ from a nonmitochondrial Ca$^{2+}$ pool.\textsuperscript{41-43} Similarly, addition of IP$_3$ to the cardiac sarcoplasmic reticulum vesicles was also reported to induce Ca$^{2+}$ release.\textsuperscript{3} Thus, a reduced number of $\alpha_1$-adrenergic receptors in the myocardium of SHR is likely to cause a decrease in Ca$^{2+}$ influx across sarcolemma or Ca$^{2+}$ release from the intracellular Ca$^{2+}$ pools during excitation-contraction coupling in response to $\alpha_1$-adrenergic receptor stimulation due to 1) a decrease in IP$_3$ production, 2) Ca$^{2+}$-dependent Ca$^{2+}$ release, or 3) a decrease in the intracellular Ca$^{2+}$ storage capacity of sarcoplasmic reticulum.\textsuperscript{44}

In the myocardium, transmembrane Ca$^{2+}$ influx during action potential is controlled by opening of the slow Ca$^{2+}$ channels on depolarization of the cell. The extent of slow-channel opening is also controlled by activation of cyclic adenosine 3',5'-monophosphate (cAMP)-dependent protein kinase and the phosphorylation of protein at the phosphorylation-dependent Ca$^{2+}$ release, or 3) a decrease in the intracellular Ca$^{2+}$ storage capacity of sarcoplasmic reticulum.\textsuperscript{44}

![Figure 6. Contractile protein myosin ATPase activity in the myocardium of SHR and WKY. A. Actin-activated Mg$^{2+}$-ATPase activity of purified myosin (actin/myosin molar ratio, 15:1); B. Ca$^{2+}$-Mg$^{2+}$ ATPase activity of myofibrils. C. Ca$^{2+}$-ATPase activity of actomyosin (0.05 M KCl, 10 mM CaCl$_2$). D. Ca$^{2+}$/EDTA-ATPase activity of purified myosin (0.5 M KCl, 4 mM EDTA); E = Ca$^{2+}$-ATPase activity of purified myosin (0.5 M KCl, 10 mM CaCl$_2$). The ATPase assays were performed at 30°C while ATP hydrolysis was between 5 and 20% as described in Methods. Results are mean ± SEM of four separate experiments. Pi = inorganic phosphate.](http://hyper.ahajournals.org/content/589/6/589.full.png)
There are three potential mechanisms for increasing cytosolic Ca\(^{2+}\) concentration in cardiac muscle: 1) the slow inward Ca\(^{2+}\) current regulated by membrane potential changes, \(\alpha\)-adrenergic receptor stimulation, and cAMP-dependent protein phosphorylation; 2) Na\(^{+}\)-Ca\(^{2+}\) exchange across sarcolemma, which can be regulated by alterations in Na\(^{+}\)-K\(^{+}\)-ATPase activity; and 3) Ca\(^{2+}\) release from sarcoplasmic reticulum, either by Ca\(^{2+}\)-dependent Ca\(^{2+}\) release triggered by transmembrane Ca\(^{2+}\) influx or by IP\(_3\)-mediated Ca\(^{2+}\) release. Our results indicate that \(\alpha\)-adrenergic and \(\beta\)-adrenergic receptor-mediated mechanisms for intracellular Ca\(^{2+}\) regulation are attenuated in the myocardium of SHR. To compensate for this decrease, Na\(^{+}\)-Ca\(^{2+}\) exchange and membrane-depolariization-dependent Ca\(^{2+}\) influx appear to be increased in the myocardium of young adult SHR. However, from these results it is not clear whether these changes in the myocardium of SHR are associated with the development of hypertension or are inherited defects. It will be of interest to investigate these mechanisms in prehypertensive animals and after treatment of these animals with antihypertensive drugs to establish whether these changes are a consequence of the hypertensive process or represent a contributory cause.

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