Decrease in Na⁺,K⁺-ATPase Activity and [³H]Ouabain Binding Sites in Sarcolemma Prepared from Hearts of Spontaneously Hypertensive Rats

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SUMMARY Na⁺,K⁺-ATPase activity, phosphorylation, and [³H]ouabain binding in sarcolemma isolated from spontaneously hypertensive rat (SHR) hearts were compared to the same parameters in sarcolemma from normotensive rat (WKY) hearts. Sarcolemma prepared from SHR heart contained significantly less ouabain-inhibitable ATPase activity than sarcolemma from WKY heart. No significant differences in sarcolemmal protein content or recovery were noted between the two groups. The numbers of phosphorylation sites and ouabain binding sites were lower for SHR hearts than for WKY hearts. The Kᵦ values for ouabain binding were the same (0.30 µM) in cardiac sarcolemma of SHR and WKY. The I₅₀ values for inhibition by ouabain of Na⁺,K⁺-ATPase were also the same for both groups (SHR = 49 µM; WKY = 44 µM). These data suggest that the decrease of cardiac sarcolemmal Na⁺,K⁺-ATPase activity in SHR hearts is due to a decrease in the number of active sites.

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KEY WORDS • sodium pump • hypertension • Na⁺,K⁺-ATPase • ouabain

Since Tobian and Binion 1 reported that increased sodium and water content of the arterial wall is associated with hypertension, it has been suggested that inhibition of the vascular smooth muscle Na⁺,K⁺ pump might be connected with several types of hypertension. The possible role of Na⁺,K⁺-ATPase in control of vascular smooth muscle tone was suggested by Chen et al. 2 Overbeck 3 was the first to speculate that a defect in the sodium pump of vascular smooth muscle might be associated with hypertension. Subsequent reports in support of this suggestion have appeared. 4-6 Additional evidence for this suggestion derives from experiments showing decreased ouabain sensitive ⁴⁶Rb uptake (presumed to be a measure of Na⁺,K⁺ pump activity) in isolated mesenteric vessels of renal hypertensive dogs ⁸ and in tail arteries of deoxycorticosterone acetate-treated hypertensive rats, ⁹ as well as reports of decreased Na⁺,K⁺-ATPase activity in microsomes from hearts of renal hypertensive rats ¹⁰ and in brain microsomes from uremic rats. ¹¹

The spontaneously hypertensive rat (SHR) is one model for use in the study of human essential hypertension. Although the mechanism of the development of spontaneous hypertension in the rat has not been fully elucidated, abnormal ion transport across the vascular smooth muscle membrane may play some role or represent one pathophysiological characteristic. ¹² For example, a decrease of sodium pumping in hypertension could lead to an increase in intracellular sodium, which in turn would cause a net increase in internal calcium with a resultant increased peripheral vascular resistance. Because Na⁺,K⁺-ATPase is the membrane enzyme primarily responsible for Na⁺ and K⁺ transport in most animal cells, ¹³ abnormal ion transport in SHR could be due to changes in certain properties of this enzyme. It has been reported that in SHR and in humans with essential hypertension, the left ventricle becomes progressively hypertrophic and heart rate may be elevated at all stages. ¹⁴ These changes in the myocardium may conceivably result from alterations in Na⁺,K⁺ pump activity of the myocardium.

In the present study, we examined Na⁺,K⁺-ATPase activity, sodium-dependent phosphorylation, and the interaction of ouabain with this enzyme in a highly enriched preparation of cardiac sarcolemma prepared from ventricular myocardium of SHR and WKY.
Methods

Spontaneously hypertensive rats (SHR), control normotensive Wistar-Kyoto (WKY) rats, and unrelated Wistar rats were obtained from Laboratory Supplies, Indianapolis, Indiana, and from the laboratory of Dr. Yamori of Shime University, Izumo, Japan. Blood pressure was measured by the tail-cuff method. The rats were decapitated and their hearts were rapidly removed, blotted, and weighed. Cardiac sarcolemma was prepared identically for each group of rats by a modification of the method described by Van Alstyne et al. After weighing 20 to 45 individual hearts, the atria, connective tissue, and major vessels were removed, and hearts were pooled and weighed. The tissue was then minced and homogenized using a Polytron PT-20; this was done twice for 5 seconds at one-half maximum speed in 5 volumes of a solution containing 10 mM NaHCO₃, 5 mM NaN₃, and 0.25 M sucrose at a pH of 7.0. Samples of the homogenate were taken and analyzed for enzyme activities. Protein content was estimated by the method of Lowry et al. Microsomes were prepared by differential centrifugation exactly as described by Van Alstyne et al. Cardiac membranes highly enriched in sarcolemma were then isolated by discontinuous sucrose gradient centrifugation of the microsomal suspension, and the final sarcolemma fraction was suspended in 10 mM Tris-HCl, pH 7.4. The average yield of sarcolemma membranes isolated from 1 g of tissue was 138 μg for both the SHR and WKY hearts. A typical preparation from WKY exhibited ouabain-sensitive Na⁺,K⁺-ATPase activity of 130 μmol/mg/hr, phosphorylation sites of 241 pmol/mg, [³H]dihydropyridine benzilate binding sites of 0.66 pmol/mg, and [³H]quinuclidinyl benzilate binding sites of 2.7 pmol/mg.

The relative purity of the sarcolemma-enriched membrane fraction and its ATPase content were analyzed using a linked-enzyme spectrophotometric assay previously described. Total and specific ATPase activities of both SHR and WKY rat cardiac sarcolemma were measured in a reaction medium containing 100 mM NaCl, 10 mM KCl, 5 mM MgCl₂, 5 mM Na₂ATP (vanadium-free; Boehringer), 0.4 mM NADH, 1 mM phosphoenolpyruvate, 14 units pyruvate kinase plus 20 units lactate dehydrogenase (supplied by Sigma Chemical Company, St. Louis, Missouri) and 25 mM L-histidine, pH 7.2. After equilibration of the medium at 37°C, 5–10 μg (protein) of sarcolemma (or 20–30 μg of homogenate, which had been previously diluted 20–30 times in 10 mM Tris-Cl, pH 7.4) was added to a final volume of 2.5 ml to initiate the ATPase reaction. Enzyme activity was determined by continuous monitoring of the decrease in absorbance at 340 nm due to the oxidation of NADH. In the absence of inhibitors (see below), this determination is a measure of total ATPase activity. Mitochondrial ATPase activity was estimated by measuring the difference between total ATPase activity and the activity remaining 30 minutes after the addition of ouabain (1 mM final). This concentration of ouabain was required to fully inhibit rat cardiac Na⁺,K⁺-ATPase as determined from concentration-inhibition curves (see below). A residual, Mg²⁺-ATPase activity was measured after the further addition of EGTA (0.1 mM final), which also represented a basal ATPase activity for the determination of calcium-dependent ATPase. After the addition of CaCl₂ to the cuvette (up to 0.15 mM), the ATPase activity expressed minus the basal Mg²⁺-ATPase activity represented the Ca²⁺-ATPase activity that was fully inhibited by 1.0 mM EGTA.

Sodium-dependent phosphorylation of Na⁺,K⁺-ATPase was carried out at 0°C by a filtration method, as previously described. The reaction medium contained 3 mM MgCl₂, 25 mM histidine (pH 7.4), 20 mM NaCl, and 50 μg of enzyme in a volume of 0.5 ml. The reaction was initiated by the addition of γ[³P]ATP (40 μM) and terminated after 10 seconds by the addition of 3 ml of an ice-cold wash solution containing 10% trichloroacetic acid, 0.6 mM Na₂ATP, and 0.6 mM H₃PO₄. The tubes were immediately placed in an ice bath, and the contents filtered through 0.45 μm Gelman filters. The filters were washed three times with 5 ml of the ice-cold wash solution and placed in 8 ml of scintillation fluid for 12 hours before counting in a Beckman LS 200B liquid scintillation counter. Non-specific binding was measured by the inclusion of 5 mM unlabeled ATP in the above medium and was subtracted from the total.

Binding of [³H]ouabain (19.5 Ci/mmol; New England Nuclear Corporation, Boston, Massachusetts) was carried out by the filtration method as previously described except that a displacement assay was used to determine the apparent Kₐ, as a measure of the affinity of Na⁺,K⁺-ATPase for ouabain. Ouabain binding was carried out in the presence of 100 mM NaCl, 10 mM KCl, 5 mM MgCl₂, 5 mM Na₂ATP, 25 mM histidine, 0.4 mM NADH, and 1 mM phosphoenolpyruvate. After incubation of sarcolemmal membranes (100 μg/ml) for 90 minutes at 37°C with 0.1 μM [³H]ouabain and various concentrations of unlabeled ouabain (as indicated), the reaction mixture was rapidly filtered on 0.45 μm methylcellulose filters (Gelman). Preliminary experiments indicated that equilibrium binding was obtained in 30 minutes at 37°C in the presence of 0.1 μM [³H]ouabain. The filters were then washed three times with 5 ml of cold 100 mM KCl and incubated at least 10 hours in scintillation fluid (Budgetsolv) prior to determination of radioactivity.

The ouabain binding site concentration and affinity were estimated as described previously by Akera and Cheng. The binding of a fixed concentration of [³H]ouabain (0.1 μM) was assayed in the presence of various concentrations of the unlabeled ouabain. Non-specific ouabain binding observed in the presence of 5.5 mM unlabeled ouabain was subtracted from values observed in its absence to calculate saturable [³H]ouabain binding. The apparent Kₐ and Bₐₘ were calculated by the following equations:
TABLE 1. Blood Pressures, Body Weights, Heart Weights, and Protein Contents of Cardiac Homogenate and Membrane Fractions

<table>
<thead>
<tr>
<th>Rat group</th>
<th>Age (wks)</th>
<th>No. of animals</th>
<th>WKY</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>W</td>
<td>S</td>
</tr>
<tr>
<td>1</td>
<td>18</td>
<td>90</td>
<td>129</td>
<td>168*</td>
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<tr>
<td>2</td>
<td>16</td>
<td>29</td>
<td>138</td>
<td>195*</td>
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<td></td>
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<td>±2</td>
<td>±2</td>
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<td>3</td>
<td>19</td>
<td>31</td>
<td>133</td>
<td>192*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>±2</td>
<td>±2</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>45</td>
<td>108</td>
<td>185*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>±3</td>
<td>±3</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>± 7 ± 6</td>
<td>127</td>
<td>185*</td>
</tr>
</tbody>
</table>

*Significantly different from corresponding group.
†Means ± SEM are from four different preparations of WKY and SHR each.
§Sarcolemmal protein recovery from homogenate.

Rat groups 1, 2, 3 were obtained from Laboratory Supplies, Indianapolis, Indiana. Group 4 was obtained from Dr. Yukio Yamori of Shimane Medical University, Izumo, Japan.

K₀ = C₀,5 - a
Bmax = B₀C₀,5/a

where C₀,5 is the concentration of unlabeled ouabain that caused a 50% reduction of the previously bound labeled ouabain, a is the labeled ouabain concentration, and B₀ is the equilibrium binding of the labeled ouabain in the absence of unlabeled ouabain.

Another measure of the affinity of the Na⁺,K⁺-ATPase for ouabain was made by determining the concentration of ouabain which inhibited 50% of the specific Na⁺,K⁺-ATPase activity (I₀). Sarcolemmal membranes (~20 μg) were incubated in a medium containing 20 mM histidine (pH 7.2), 100 mM NaCl, 10 mM KCl, 5 mM MgCl₂, 5 mM Na₃ATP, 0.4 mM NADH, 1 mM phosphoenolpyruvate, 14 units pyruvate kinase, 20 units lactate dehydrogenase, and increasing concentrations of ouabain (10⁻² M to 7.8 x 10⁻⁴ M). Tris-EGTA (0.1 mM) and 5 mM NaN₃ were included with simultaneous measurement of control Na⁺,K⁺-ATPase activity and azide-sensitive ATPase activity (see above). For all sarclemma preparations from WKY and SHR hearts, measurements of the inhibition by ouabain of enzyme activity were made in triplicate at each dose of ouabain with simultaneous measurement of control Na⁺,K⁺-ATPase activity in the absence of ouabain. Enzyme activity was continuously monitored for 30 minutes at 37°C by the spectrophotometric method, and the apparent steady-state, ouabain-inhibitable Na⁺,K⁺-ATPase activity was measured and plotted as the percentage inhibition of the control activity. The I₀ value for ouabain was then determined by graphical analysis of the concentration-inhibition curves.

Statistical analysis was performed with Student's t test, and the level for significance was taken as a probability less than 5% (p<0.05). All values reported represent the means ± SEM.

Results

WKY and SHR were matched according to strain and age, and four different groups of rats were studied (table 1). The blood pressures of the SHR were significantly increased over those of the WKY controls in all groups. The SHR of Group 1 had lower blood pressures compared to the SHR in the other groups. The total body weights of WKY in Group 1 were the same as for SHR. Whole heart weights showed a slight tendency toward hypertrophy, but this was not statistically significant. SHR Groups 2 and 3 showed significant increases in both body weight and heart weight. The ratio of total heart weight-to-total body weight was increased in SHR Group 2 but surprisingly decreased in SHR Group 3. The animals in SHR Group 4 (from the laboratory of Dr. Yamori) showed a significant decrease in body weight and a significant increase in heart weight. Therefore, the heart-to-body-weight ratio was increased, indicating a substantial cardiac hypertrophy in these rats. If one combined all four groups, the only significant difference was the elevated systolic blood pressure in the SHR. The homogenates from hearts of SHR showed a tendency to contain more protein per gram of tissue compared to WKY, but this tendency was not statistically significant. The yield of sarcolemmal protein per gram of tissue was almost the same for the WKY and SHR, although the recovery of sarcolemmal protein from the homogenate was 3% to 12% greater in WKY.

Enzyme activities of four separate membrane preparations from Group 1 rats are shown in table 2. In homogenates, azide-sensitive ATPase activity and Na⁺,K⁺-ATPase activity were both decreased. In sarclemma prepared from SHR hearts only the Na⁺,K⁺-ATPase activity was significantly decreased. Compared with preparations from WKY hearts, the Na⁺,K⁺-ATPase activity was 41% lower in SHR cardiac homogenates and 35% lower in the SHR sarcolemmal-enriched fraction. This reduction in Na⁺,K⁺-
ATPase activity was apparently not related to the degree of hypertrophy (table 3). In two separate sarcolemmal-enriched preparations from unrelated Wistar rats, the ouabain-sensitive ATPase activity was 128 μmol Pi/mg protein/hr, which is very similar to the degree of hypertrophy (table 3). In two separate sarcolemmal-enriched preparations from hearts of WKY and SHR, the ouabain-sensitive ATPase activity was 128 μmol Pi/mg protein/hr, which is very similar to the degree of hypertrophy (table 3). In two separate sarcolemmal-enriched preparations from hearts of WKY and SHR, the ouabain-sensitive ATPase activity was 128 μmol Pi/mg protein/hr, which is very similar to the degree of hypertrophy (table 3). In two separate sarcolemmal-enriched preparations from hearts of WKY and SHR, the ouabain-sensitive ATPase activity was 128 μmol Pi/mg protein/hr, which is very similar to the degree of hypertrophy (table 3).

Sodium-dependent phosphorylation of Na⁺,K⁺-ATPase from gamma-labeled [³²P]ATP was used to determine the number of active sites of the enzyme present in the sarcolemmal preparations. The number of phosphorylation sites in sarcolemma prepared from SHR hearts was 37% lower than sarcolemma prepared from hearts of WKY or unrelated Wistar rats (table 3). The turnover number (the ratio of enzyme activity to phosphorylation sites) was the same in preparations from hearts of WKY or unrelated Wistar rats (table 3).

The concentration of ouabain which half-maximally inhibits enzyme activity was the same (I₅₀ = 50 μM) for sarcolemma prepared from the SHR and WKY hearts (fig. 1).

In rat ventricular myocardium, there appear to be two different classes of Na⁺,K⁺-ATPase with respect to their different affinities for ouabain. One site, char-

<table>
<thead>
<tr>
<th>Table 1. (Continued)</th>
<th>Table 2. ATPase Activities of Cardiac Preparations from SHR and WKY</th>
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<tbody>
<tr>
<td>Homogenate Sarcolemma</td>
<td>Enzyme activity (μmol Pi/mg protein/hr)</td>
</tr>
<tr>
<td>Homogenate Sarcolemma</td>
<td></td>
</tr>
<tr>
<td>WKY (mg/wks)</td>
<td>SHR (mg/wks)</td>
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<tr>
<td>WKY</td>
<td>SHR</td>
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<tr>
<td>41.0</td>
<td>47.1</td>
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<tr>
<td>36.3</td>
<td>37.9</td>
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<tr>
<td>37.8</td>
<td>42.6</td>
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<td>40.0</td>
<td>44.0</td>
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<tr>
<td>38.8</td>
<td>42.9</td>
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<tr>
<td>±1.1</td>
<td>±1.9</td>
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</tbody>
</table>

1. The groups are the same as described in Table 1. Measurement of Na⁺,K⁺-ATPase, phosphorylation and [³H]ouabain binding was carried out as described in Methods.

*Significantly different (p < 0.05) from corresponding value of WKY.†The sarcolemma from the unrelated Wistar strain was prepared exactly as from WKY and SHR. The preparations and measurement of enzymatic parameters were not done in parallel with the WKY and SHR.
characterized by a low apparent affinity for ouabain (50 μM) which is calculated from the I₅₀ curve described above, represents a large proportion of the total number of sites, as detected by sodium-dependent phosphorylation. Recently a [³H]ouabain displacement assay has been used to detect a site with a higher apparent affinity (Kᵦ = 0.3 μM), which may represent a small portion of the total number of sites.²⁵⁻²⁷ This displacement assay was used to compare the apparent Kᵦ values and number (B₅₀) of higher affinity sites in WKY and SHR sarcolemma. In figure 2, the binding of [³H]ouabain in the presence of unlabeled ouabain is expressed as a percentage of that measured in the absence of unlabeled ouabain (probit scale) and plotted against the unlabeled ouabain concentration (in logarithmic scale). The apparent Kᵦ values (dissociation constants), calculated from the equation shown in the Methods section, were not different in the two groups. However, in cardiac sarcolemma prepared from SHR, the binding site concentration was markedly lower (49%) than that of WKY (table 3). The characteristics of [³H]ouabain binding to sarcolemmal preparations from hearts of unrelated Wistar rats were similar to that of WKY (table 3).

Discussion

The major findings of this study are: 1) the concentration of available Na⁺,K⁺-ATPase active sites per milligram of sarcolemma is lower in hearts of SHR than in WKY; and 2) the concentrations of both classes of ouabain binding sites are reduced.

Although the heart weights of the SHR were either significantly increased or showed a tendency toward increase, we were surprised at the variation of the ratio of heart weight to average body weight in the four groups. The WKY from the laboratory of Dr. Yamori were heavier than the WKY obtained from the commercial source, and the hearts of the Yamori SHR were clearly hypertrophied. The individual heart-weight to body-weight ratios of a subgroup (n = 46) of the Group 1 rats were determined. A linear regression (six aberrant data points were excluded from the regression since their heart-weight to body-weight ratios were not in the range of mean ± 4 SD) yielded the relationship: heart weight = 146 mg + 2.77 mg/g × body weight. Applying this equation to the SHR body weights yielded a predicted heart weight for SHR hearts of 800 ± 10 mg, which was significantly different (p < 0.005) from the actual heart weights (870 ± 20 mg). Because the preparation of sarcolemma with a highly active Na⁺,K⁺-ATPase requires fresh hearts, we did not obtain these detailed data for the other groups. It is probable, though, that at least slight hypertrophy did occur in all groups. At any rate, in the present study, the degree of hypertrophy is apparently not correlated with the reduction in the sodium pump and ouabain binding sites.

Because hypertrophy causes an increase in cell volume, one might expect a decrease in the ratio of sarcolemmal protein to total protein. Our data indicate that the yield of protein in homogenates from SHR was 10% greater than from WKY, and that the recovery of sarcolemmal protein from the homogenate was slightly lower for SHR. The necessity of pooling 20 to 40 hearts for each preparation and the variability in the yield from preparation to preparation prevented any definitive conclusions about the relationship of hypertrophy to sarcolemmal protein content.

The 40% reduction in the concentration of sodium pump and ouabain binding sites cannot be accounted for by the slight differences in protein yield or by artifacts of the isolation procedure. The reduction in Na⁺,K⁺-ATPase activity clearly is not due to a decrease in the intrinsic turnover number of the enzyme,
since the enzyme activity and the number of active sites were both reduced by the same amount. The Na⁺,K⁺-ATPase activity of our sarcolemmal preparations from rat hearts is activated only about 20% by freeze-thaw procedures, suggesting that most of the vesicles are inside-out and/or leaky. It has been suggested that the ion permeability of vascular smooth muscle in SHR is increased\textsuperscript{14,15} and that the vascular smooth muscle membrane of the hypertensive rat is more labile than that of the normotensive rat.\textsuperscript{28} If the same were true in heart sarcolemmal vesicles, one would expect an increase in detectable patent Na⁺, K⁺-ATPase activity, which is the opposite of our finding.

A decrease of Na⁺, K⁺-pump activity of the same magnitude in the blood vessels and hearts of rats in which hypertension was surgically induced has been reported by Pannani et al.\textsuperscript{11} and Clough et al.\textsuperscript{12} On the other hand, there are other studies in various tissues that report Na⁺, K⁺-pump activity to be higher, lower, or no different from that of controls.\textsuperscript{29-32} This is not surprising since different models of hypertension and different organs were studied using membrane preparations of differing degrees of purity. In addition, different methods, some of which suffer from the probability of large errors, were used to estimate the number of sodium pump sites. Rubidium uptake was, for example, often used to estimate sodium pump activity. This may not be an adequate measurement of the activity or the number of pump sites, because, as Akera et al.\textsuperscript{33} have pointed out, the extent of sodium loading has an effect on Rb uptake. The present results, therefore, should be viewed within the perspective of this particular model of hypertension.

With respect to our methodology, a few comments are in order. In cardiac glycoside-sensitive species, the number of [³²P]ouabain binding sites correlates very well with the number of active sites (pump sites) of Na⁺, K⁺-ATPase. The low affinity of rat heart and kidney Na⁺, K⁺-ATPase for ouabain, however, makes the acquisition of radioligand binding data very difficult. The measurement of Na⁺, K⁺-ATPase activity is also subject to large errors in crude membrane preparations because of high ouabain-insensitive ATPase activity. For membrane preparations from rat heart, the most accurate estimation of pump sites is the measurement of sodium-dependent phosphorylation from γ[³²P]ATP. The present study demonstrates a significantly lower level of cardiac Na⁺, K⁺-ATPase activity in sarcolemma isolated from SHR, which agrees remarkably well with a decrease in phosphorylation levels as compared to cardiac sarcolemma of WKY. These data therefore strongly indicate a reduction in the density of Na⁺, K⁺-pump sites in the preparations examined.

For membrane preparations from ouabain-sensitive species, the I₅₀ value, estimated from inhibition of Na⁺, K⁺-ATPase activity after long periods of incubation with ouabain, approaches the Kᵦ value estimated by [³²P]ouabain binding and the number of phosphorylation sites equals the number of ouabain binding sites.\textsuperscript{34} In the relatively insensitive rat heart, there is a discrepancy between the Kᵦ value (0.1–0.3 μM) and I₅₀ value (40 μM)\textsuperscript{25-27} for ouabain. It has been suggested that there are both a high affinity/low capacity binding site and a low affinity/high capacity binding site for ouabain in cell membrane preparations isolated from rat heart and in intact myocytes isolated from rat heart.

It is not possible to measure the Bᵦ of the lower affinity site with [³²P]ouabain binding. Therefore, the number of lower affinity ouabain binding sites is assumed by us to be equal to the number of phosphorylation sites. In our experiments, the Kᵦ value for the higher affinity site, as determined by a [³²P]ouabain displacement method was 0.3 μM in both SHR and WKY cardiac sarcolemma. These values agree well with the Kᵦ and I₅₀ values previously reported\textsuperscript{25-27} for preparations from normotensive rats. We found no differences in Kᵦ or I₅₀ values in preparations from SHR and WKY. The number of higher affinity [³²P]ouabain binding sites in the SHR preparations, however, was reduced compared to WKY.

One possible cause for the lower levels of Na⁺, K⁺-ATPase activity in sarcolemma of SHR myocardium might be the presence in vivo of an inhibitor of the enzyme. Haddy and Overbeck\textsuperscript{35} originally proposed a relationship between a putative natriuretic hormone, perhaps secreted from the brain\textsuperscript{36} and salt-related renal hypertension.\textsuperscript{36-38} It has been suggested, e.g., that inhibition of the ouabain-sensitive Na⁺, K⁺ pump of smooth muscle membrane in salt-loaded or renal hypertensive animals is caused by circulation of this hormone in the plasma. Blaustein\textsuperscript{37} concluded that a putative natriuretic hormone may play an important role in the development of hypertension and might act as the body's 'endogenous digitalis.' While such putative factors may be important with respect to the vascular smooth muscle of certain hypertensive models, they are unlikely to play a significant role in the present results. The procedure for the sarcolemmal preparation involves multiple, large dilutions of homogenized cardiac membranes, and, unless very tightly bound, the factor would be removed during isolation. We have no information in this regard.

One might ask what relationship a decrease in the density of sodium pump sites could have with hypertension. Numerous studies have led to the concept that inhibition of the Na⁺, K⁺-ATPase results in elevated intracellular Na⁺ which, in turn, would diminish the sodium gradient for calcium extrusion and/or increase Na⁺/Ca²⁺ exchange. In any event, this results in an elevated intracellular calcium. Such a mechanism has been postulated for the inotropic action of digitalis.\textsuperscript{16} Several investigators (for review, see ref. 39) have also suggested that this mechanism might play an important role in maintaining, as well as altering, the contractile tone of various types of muscle, including myocardium and peripheral vasculature. It is possible, e.g., that reduced Na⁺, K⁺-pump activity of SHR hearts could increase the contractility of the heart via the mechanism outlined above. However, we cannot state that the alterations in cardiac Na⁺, K⁺-pump activity are in fact related to the development of hypertension or car-
diac hypertrophy in SHR. It is unknown, e.g., whether the reduced number of Na⁺-pump sites in SHR is associated with the development of hypertension itself in these animals or is a consequence of inherited pathological features that later result in a reduced pump site density. The development of the Wistar-Kyoto hypertensive strain of rats obviously has resulted in the dominance of certain genetic traits which could conceivably result in altered ribosomal or translational events and, thus, altered protein metabolism. The decrease in cardiac sarcosomnal Na⁺,K⁺-ATPase of SHR, if also present in vascular smooth muscle, may be associated with hypertension. The major questions which remain unanswered are whether a reduced number of active Na⁺,K⁺-pump sites in the hearts of SHR is a consequence of hypertension, a contributing cause to hypertension or an unrelated phenomenon.

Acknowledgments

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