Cardiovascular Contraction in Spontaneously Hypertensive Rat:  
Ca\(^{2+}\) Interaction of Myofibrils and Subcellular Membrane 
of Heart and Arterial Smooth Muscle

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Cardiac myofibrils isolated from the hearts of Okamoto-Aoki hypertensive 
rat (SHR) and normotensive Wistar rat (NR) showed essentially the same 
ATPase activity either in the absence or presence of Ca\(^{2+}\). On the other 
hand, Ca\(^{2+}\) activated ATPase activity of relaxed preparation of cardiac 
myofibrils from SHR was significantly lower than that from NR.

ATPase activity and Ca\(^{2+}\) binding ability of cardiac sarcoplasmic reticulum 
(cardiac SR) and arterial subcellular membrane (arterial SR) isolated from 
SHR and NR were measured. The maximum Ca\(^{2+}\) binding capacity of cardiac 
SR from SHR (8.80 ± 0.33 nmole/mg) was significantly lower than that 
from NR (11.95 ± 0.55). Also, cardiac SR from SHR had significantly elevated 
Ca\(^{2+}\) activated ATPase activity (0.171 ± 0.009 μmole/mg/min) compared 
with that of NR (0.100 ± 0.009). And, the maximum Ca\(^{2+}\) binding capacity 
of arterial SR from SHR (23.55 ± 0.72 nmole/mg) was significantly lower 
than that from NR (39.35 ± 4.89). ATPase activity of arterial SR from SHR 
(2.94 ± 0.18 μmole/mg/min) was significantly elevated compared with that 
of NR (2.22 ± 0.14).

Reduction of Ca\(^{2+}\) binding capacity of both cardiac and arterial SR may 
result in increase of intracellular free Ca\(^{2+}\) concentration which probably be 
one of the important factor for increase of muscular tone.

If this abnormal intracellular distribution of Ca\(^{2+}\) is associated with 
the vascular smooth muscle, it would cause the increase of vascular resistance 
resulting in hypertension.

IT SEEMS to be that depression of ATPase activity of contractile proteins is associated 
with depressed contractility in failing heart muscle! and the activity is elevated in the state of 
physical training with increased contractility? But myofibrillar ATPase activity from the heart 
of Okamoto-Aoki hypertensive rat (SHR) was not altered? One of the most depending factors 
of contractility of muscle is the intracellular ionic environment, especially the distribution of 
Ca\(^{2+}\). The activity of actomyosin in both cardiac and skeletal, and arterial muscle, is controlled by 
Ca\(^{2+}\) and in vitro contraction of muscle is regulated by the intracellular Ca\(^{2+}\) through 
troponin-tropomyosin system. Intracellular free Ca\(^{2+}\) concentration is regulated by the sarco

Key Words: 
ATPase 
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Cardiac sarcoplasmic reticulum 
Ca\(^{2+}\) binding 
Okamoto-Aoki rat 
SHR 
Hypertension 
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TABLE I  BASIC AND Ca²⁺ ACTIVATED ATPase ACTIVITY OF RELAXED PREPARATION OF CARDIAC MYOFIBRILS

<table>
<thead>
<tr>
<th>No. of Animals</th>
<th>Sex</th>
<th>Age Weeks</th>
<th>Blood Pressure mmHg</th>
<th>ATPase Activity μmoles Pi/mg/min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Basic</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ca²⁺ activated</td>
</tr>
<tr>
<td>NR 5 F</td>
<td>30–40</td>
<td>132 ± 5</td>
<td>0.063 ± 0.001</td>
<td>0.073 ± 0.004</td>
</tr>
<tr>
<td>SHR 5 F</td>
<td>30–40</td>
<td>200 ± 10*</td>
<td>0.072 ± 0.004*</td>
<td>0.053 ± 0.002*</td>
</tr>
</tbody>
</table>

Values are expressed mean ± standard error.  F: female
Significant differences are compared to NR and SHR, *p < 0.001.

TABLE II  BASIC AND Ca²⁺ ACTIVATED ATPase ACTIVITY OF CARDIAC SARCOPLASMIC RETICULUM

<table>
<thead>
<tr>
<th>No. of Animals</th>
<th>Sex</th>
<th>Age Weeks</th>
<th>Blood Pressure mmHg</th>
<th>ATPase Activity μmoles Pi/mg/min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Basic</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ca²⁺ activated</td>
</tr>
<tr>
<td>NR 6 M</td>
<td>20–25</td>
<td>130 ± 1</td>
<td>0.146 ± 0.009</td>
<td>0.100 ± 0.009</td>
</tr>
<tr>
<td>SHR 6 M</td>
<td>20–25</td>
<td>195 ± 2*</td>
<td>0.148 ± 0.009</td>
<td>0.171 ± 0.009*</td>
</tr>
</tbody>
</table>

Values are expressed mean ± standard error.  M: male
Significant differences are compared to NR and SHR, *p < 0.01, **p < 0.05.

plasmatic reticulum which exhibits ATP dependent Ca²⁺ binding in vitro. Alteration of the ability of sarcoplasmic reticulum to bind Ca²⁺ may result in abnormal distribution of intracellular Ca²⁺. Depression of ATPase activity of the subcellular system (sarcoplasmic reticulum) and sarcolemmαβ and abnormal distribution of intracellular Ca²⁺ in muscle from failing heart7 has been demonstrated. And, our preliminary work8 on microsomes from the hearts of SHR showed the depressed Ca²⁺ binding ability.

Considerable amount of evidences have been accumulated that the contraction of smooth muscle9 as well as that in skeletal muscle, is controlled by the intracellular free Ca²⁺ concentration10,11. Ca²⁺ binding ability of subcellular membrane such as sarcoplasmic reticulum and Ca²⁺ influx through cell membrane may be an essential factor for regulation of intracellular Ca²⁺ concentration and therefore muscle tone in vascular system.

As Bohr and Sitrin12 stated, if hypertension is induced by increase of vascular tone, reduction of function of relaxing system may be expected. To clarify the mechanism of development of hypertension, 1. ATPase activity and Ca²⁺ binding ability were measured on sarcoplasmic reticulum obtained from the hearts (cardiac SR) of SHR. Also, measurement of ATPase activity of myofibrils from the heart muscle was performed. 2. The propaties of subcellular membrane isolated from vascular smooth muscle (Arterial SR), i.e., its Ca²⁺ binding ability and ATPase activity were examined in SHR and normotensive Wistar rat (NR).

MATERIALS AND METHODS

SHR was inbred in our laboratory from F₁₄ to F₂₄ in 1973. Systolic blood pressure of the rats were measured weekly by the tail cuff using water plethysmograph without anesthesia. All rats were fed with commercial chow (Nippon Clea, CE-2) and given tap water to drink.

Cardiac myofibrils were prepared by the method of Fanburg, et. al13,14 Relaxed myofibrils were prepared by perfusion of the heart with Ca²⁺ free Krebs Ringer solution containing 0.2 mM EGTA before removal of the heart for 15 min according to the method of Takauji and Honig15. Electrononmicrographs of this myofibrils

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TABLE III  MAXIMUM Ca\textsuperscript{2+} BINDING AND Ca\textsuperscript{2+} BINDING CONSTANT OF CARDIAC SARCOPLASMIC RETICULUM

<table>
<thead>
<tr>
<th>No. of</th>
<th>Sex</th>
<th>Age</th>
<th>Maximum Binding</th>
<th>Binding Constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiments</td>
<td></td>
<td>Weeks</td>
<td>nmoles Ca\textsuperscript{2+}/mg</td>
<td>10\textsuperscript{6} Ca M\textsuperscript{-1}</td>
</tr>
<tr>
<td>NR 10</td>
<td>M</td>
<td>12-20</td>
<td>11.95 ± 0.55</td>
<td>0.071 ± 0.005</td>
</tr>
<tr>
<td>SHR 8</td>
<td>M</td>
<td>12-20</td>
<td>8.80 ± 0.33*</td>
<td>0.089 ± 0.007**</td>
</tr>
</tbody>
</table>

Values are expressed mean ± standard error.  M: male
Significant differences are compared to NR and SHR, *p < 0.01, **p < 0.05.

TABLE IV  SUBCELLULAR MEMBRANE OF ARTERIAL SMOOTH MUSCLE

<table>
<thead>
<tr>
<th>Weeks of Age</th>
<th>Maximum Ca\textsuperscript{2+} Binding</th>
<th>Ca\textsuperscript{2+} Binding Constant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nmoles/mg</td>
<td>10\textsuperscript{3} [M\textsuperscript{-1}]</td>
</tr>
<tr>
<td>NR (5)</td>
<td>39.35 ± 4.89</td>
<td>12  38.65 ± 2.16</td>
</tr>
<tr>
<td>SHR (5)</td>
<td>23.55 ± 0.72</td>
<td>12  23.45 ± 0.36</td>
</tr>
<tr>
<td></td>
<td>20-40</td>
<td>20-40</td>
</tr>
<tr>
<td></td>
<td>3.34 ± 0.79</td>
<td>5.27 ± 0.43</td>
</tr>
<tr>
<td></td>
<td>10.88 ± 1.23</td>
<td>9.31 ± 0.61</td>
</tr>
<tr>
<td>p &lt;</td>
<td>0.001</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Values are expressed mean ± standard error.  (): Numbers of experiments.

revealed partly relaxed having a regular structure of both thick and thin filaments.

Cardiac myofibrillar ATPase activity was measured by determining liberated inorganic phosphate using stannous chloride as the reducing agent. Standard reaction mixture for ATPase activity of myofibrils was: 0.1 M tris-HCl, 5 mM MgCl\textsubscript{2}, 5 mM Na\textsubscript{2}H\textsubscript{2}PO\textsubscript{4}, pH 7.5, in the presence of 1 mM Ca\textsuperscript{2+} (Total ATPase) or 1 mM EGTA (Basic ATPase) and that of cardiac SR was: 0.1 M tris-HCl, 0.12 M KCl, 0.3 mM MgCl\textsubscript{2}, and 2.5 mM ATP, pH 7.5, in the presence of 0.1 mM Ca\textsuperscript{2+} or 1 mM EGTA. Ca\textsuperscript{2+} activated ATPase activity was defined as the differences between the basic and the total activity.

The cardiac SR samples for Ca\textsuperscript{2+} binding were obtained by the method of Inesi et al. The medium contained 50 mM KCl, 4 mM MgCl\textsubscript{2}, 2 mM ATP, and 4.3, 13.0 and 26.0 μM CaCl\textsubscript{2}, containing 45Ca(0.6 μCi) in 20 mM tris-maleate buffer, pH 6.4. The maximum Ca\textsuperscript{2+} binding (number of binding sites) and binding constants were calculated from the double reciprocal plot of bound and free Ca\textsuperscript{2+} and the concentrations determined by birefringent method were from 0.20 to 0.59 mg/ml and from 0.22 to 0.35 mg/ml for myofibrillar ATPase, and microsomal (Cardiac SR) ATPase, Ca\textsuperscript{2+} binding, respectively.

Subcellular membrane fractions of arterial smooth muscle (arterial SR) were prepared from the aorta, carotid, renal, iliac, and femoral arteries of the rats. Electron micrograph of the sample of arterial SR showed vesicular structure of which the most parts were smooth endoplasmic reticulum. The medium for ATPase activities were: 100 mM KCl, 3 mM ATP, 3 mM MgCl\textsubscript{2} and 10 mM tris-HCl (pH 7.4) in the presence of 0.1 mM Ca\textsuperscript{2+} or 2 mM EGTA. Ca\textsuperscript{2+} binding were measured by centrifuge method in the same buffer containing Ca\textsuperscript{2+} 2.0 x 10\textsuperscript{-5} 5.0 x 10\textsuperscript{-5} and 8.0 x 10\textsuperscript{-5} or 1.6 x 10\textsuperscript{-4}M with 45Ca(0.6 μCi). The maximum Ca\textsuperscript{2+} binding (number of binding sites) and binding constant of arterial SR were calculated as same as those of cardiac SR. Values were expressed mean ± standard error of mean.

RESULTS

Mean arterial systolic blood pressure of NR and SHR in male was 105 ± 5.2 and 143 ± 3.3 mmHg, respectively, at five weeks of age, and the blood pressure of SHR was significantly higher than that of the NR (p < 0.001). The pressures
of SHR increased with age reaching approximately 200 mmHg in male, and 180 mmHg in female at 15 weeks of age, but that of NR remained approximately 125 mmHg. The heart weight to body weight (per 100 g) ratio of SHR (264 ± 5 mg) was significantly higher than that of NR (320 ± 8) (p < 0.01) respectively, at 8 to 13 weeks of age.

There were no significant differences in ATPase activity of cardiac myofibrils between NR (0.220 ± 0.007 μmoles/mg/min) and SHR (0.206 ± 0.010). The activities were inhibited by the addition of 5 mM NaN₃, probably due to the mitochondrial contamination. Ca²⁺ activated ATPase activities also showed no significant differences. In the relaxed preparation of myofibrils from SHR showed significantly higher basic ATPase activity (0.072 ± 0.004 μmoles/mg/min) and lower Ca²⁺ activated ATPase (0.053 ± 0.002), comparing with those from NR (0.063 ± 0.001, and 0.073 ± 0.004, respectively) (Table I).

Basic ATPase activity of cardiac SR from NR and SHR showed no significant differences and not inhibited by 5 mM sodium azide. The Ca²⁺ activated ATPase activity of cardiac SR from SHR was significantly higher than that from NR (Table II). The number of binding sites in cardiac SR from SHR was significantly lower comparing with that from NR (p < 0.01), and the binding constants from SHR was higher than that from NR (p < 0.005) (Table III).

Arterial weight (mg) to body weight per 100 g at 12 weeks of age was 45 ± 3.3 in NR and 48 ± 0.7 in SHR i.e., there was no significant differences between SHR and NR.

ATPase activity of arterial SR from NR was 2.22 ± 0.14 μmoles/mg/min, and that from SHR was 2.94 ± 0.18 μmoles/mg/min, in the presence of 5 mM NaN₃ without Ca²⁺ (2 mM EGTA). The NaN₃ inhibited ATPase activity about 30% indicating the presence of mitochondrial contamination. Ca²⁺ binding ability by arterial SR from SHR (maximum binding capacity) was significantly lower than that from NR. On the other hand, Ca²⁺ binding constant by arterial SR was higher in SHR than that of NR. (Table IV).

**Discussion**

Okamoto-Aoki rats (SHR) developed hypertension genetically as reported in 1973. The blood pressure of SHR were significantly higher than that of NR from 5 weeks of age in NIH laboratories as well as in our laboratory. Cardiac hypertrophy always occured after 8 weeks.

The total and basic ATPase activity of cardiac myofibrils of NR and SHR were the same throughout all ages. Different from failing heart, the cardiac myofibrillar ATPase activity per protein in SHR is not altered. Because of cardiac hypertrophy, the total and basic ATPase activity per heart in SHR is higher than that in NR. In order to maintain blood circulation against increased vascular resistance in hypertension, extra energy for the extra work against elevated blood pressure would be supplied by the increased mass of heart muscle.

Ca²⁺ activated ATPase activity of myofibrils in the presence of Mg²⁺ at low ionic strength can be considered as the one which responsible for contraction of myofilaments. The lower Ca²⁺ activated ATPase activity of relaxed preparation of myofibrils (although it is not completely relaxed) from the heart of SHR may be due to one of the following reasons: 1) ATPase activity of contractile elements (actomyosin) per protein is reduced in SHR, the extent of reduction is small, however. 2) Activity of tropomysosin (inhibition of actomyosin ATPase) is reduced, so that activation of myofibrillar ATPase activity by Ca²⁺ is apparently small. 3) Proteins other than actomyosin, such as respiratory enzymes, increased in hypertrophied heart.

Cardiac SR seems to have similar action to that in skeletal muscle as a relaxing system by regulating intracellular free Ca²⁺ concentration in skeletal muscle in vivo. Ca²⁺ activated ATPase activity of the cardiac SR was diminished in the heart during failure and hypothyroidism, however, it was increased in the heart with hyperthyroidism. The Ca²⁺ binding capacity of cardiac SR is consistant with the role of cardiac muscle relaxation especially the duration of the active state and the rate of relaxation. Ca²⁺ uptake ability of cardiac SR from failing heart was reduced, however the rate of Ca²⁺ uptake was increased in the heart with hyperthyroidism. These results suggest there are some defects in the regulatory system of intracellular Ca²⁺ distribution in the heart both in failure and hyperthyroidism.

Both cardiac and arterial SR from SHR exhibited low Ca²⁺ binding capacity (low maximum binding) with slightly increased binding constant, and its Ca²⁺ activated ATPase activity was significantly higher than that of NR. The low Ca²⁺ binding associated with high ATPase activity of cardiac and arterial SR from SHR may reflect...
decoupling occurred between Ca\(^{2+}\) uptake and energy supply. Also, the subcellular membrane (both cardiac and arterial SR), like sarcoplasmic reticulum, from SHR would be leakier, so that it cannot accumulate Ca\(^{2+}\) in high level, although the Ca\(^{2+}\) pump would maintain in high rates. Therefore, intracellular free Ca\(^{2+}\) concentration may be increased by low Ca\(^{2+}\) binding of cardiac SR and arterial SR in SHR.

The reduction of function of relaxing system, subcellular membrane and/or the increase of Ca\(^{2+}\) influx of cell membrane, through increase of intracellular free Ca\(^{2+}\), possibly induce high tone of vascular smooth muscle. This mechanism would cause the increase of resistance in blood circulation, and then resulting in increase of blood pressure, which is hypertension, as a compensatory process of reduction of function of relaxing system.

REFERENCES


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Fig. 1.

**Comparison of Ca**

**Uptake**

**by Cardiac & Arterial Microsomal Vesicles**

**in a Medium Containing 40 μM of Ca**

**Fig. 2.**

how an increase in peripheral vascular resistance at an initial stage of the hypertension is induced. In this paper, I think, Dr. Aoki has attempted to clarify this problem from biochemical standpoint concerning contraction — relaxation mechanism in cardiovascular system.

Prior to general discussion for Dr. Aoki's paper, I would like to present our data concerning a role of the microsomal vesicles from normotensive canine arteries for contractility of Ca**2+**-sensitive arterial natural actomyosin (myosin B) firstly extracted by us (J. Jap. Coll. Angiol., 9: 176, 1969; Jap. Circulation J., 37: 229, 1973), because we have already reported some experimental findings regarding to the above problem (Fed. Proc., 26: 598, 1967; Jap.
As shown in Fig. 1, the arterial microsomes being composed of vesicular fragments with electron microscopy accumulated calcium in the presence of ATP and Mg\(^{2+}\) at 37°C. The total calcium uptake was proportional to amounts of the microsomal vesicles added, but the calcium uptake, as shown in Fig. 2, was markedly slower and weaker than that of cardiac microsomal vesicles. Under the optimal condition, the maximal calcium accumulation capacity of arterial microsomes was about 0.033 μ moles per mg microsomal protein in the medium containing 40 μ moles of CaCl\(_2\) per ml.

If the arterial microsomes were capable of inducing relaxation of arterial smooth muscle through their action of taking up free Ca\(^{2+}\) necessary for contraction of arterial smooth muscle, at least, it should be expected to demonstrate that an addition of the arterial microsomes to a reaction mixture containing Ca\(^{2+}\) being sufficient for induction of superprecipitation of the myosin B from arterial smooth muscle resulted in an inhibition of the superprecipitation. This was demonstrated in the experiment shown in Fig. 3. That is, the addition of the microsomes to the reaction mixture with myosin B, MgCl\(_2\), CaCl\(_2\), ATP and ATP-generating system consisting of phosphoenolpyruvate (PEP) and pyruvate kinase (py-k) inhibited the superprecipitation of the myosin B, although a grade of inhibition of the superprecipitation by addition of the microsomes appeared to be somewhat greater than that expected from the capacity of the microsomes taking up Ca\(^{2+}\) in the reaction mixture.

In this connection with regards to Ca\(^{2+}\) on superprecipitation, the evidence that calcium uptake by arterial microsomes from SHR was lower than that from normotensive rats found by Dr. Aoki might be tentatively interpreted that arterial muscular tone in SHR being responsible for vascular resistance was higher than that in normotensive ones, if constancy of the amounts of the microsomes and the intracellular Ca\(^{2+}\) concentration exposed to the microsomes is met between them and there is no difference between the contractile activities of arterial myosin B of SHR and normotensive rats.

As shown in Fig. 4, we have found that the pattern in disc electrophoresis of arterial microsomal protein solubilized with 1% SDS (sodium dodecyl sulfate) exhibited some differences between the microsomes from SHR and from normotensive rats. Therefore, I think there may be some qualitative differences between arterial microsomes of SHR and those of normotensive rats.

Dr. AOKI: Thank you for your suggestions. There may be a possibility that qualitative abnormality of the microsome of SHR may produce to reduce Ca\(^{2+}\) binding of arterial microsome of SHR.

Dr. UCHIDA (The Cardiovascular Research Institute): I think a behavior of influx of the extracellular Ca\(^{2+}\) into the intracellular space in the resistance vessel should be considered in the study investigated by Dr. Aoki with regard to the etiology of SHR, because the influx of extracellular Ca\(^{2+}\) is considered to be an important one among some factors providing the level of vascular resistance.

Dr. AOKI: I think it is very important and very interesting. Such an investigation should be undertaken. Thank you Dr. Uchida.

Dr. YAMORI (Kyoto Univ.): In a study on the arterial microsomal protein of SHR, rats belonging to the lineage of Wistar-Kyoto genetically close to SHR must be used as control, because we have already found some differences in an pattern of isozyme of an enzyme even between subdivisions of SHR. Furthermore, the study of etiology of hypertension based on a genetical view point must be performed not only F\(_1\) of SHR and the normotensive rat, but in F\(_2\) of them, and the correlation between the level of hypertension and the abnormalities of features of the protein should be investigated.

Dr. SHIBATA: I would like to have the Wistar-Kyoto rat. Please supply to us. Thank you Dr. Yamori.