Cardiac Hypertrophy in Spontaneously Hypertensive Rats

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SUMMARY

The energy metabolism of cardiac hypertrophy in spontaneously hypertensive rats (SHR) was studied chronologically by histochemical and in part chemical methods. The activities of various enzymes, such as glucose-6-phosphate dehydrogenase (G6PDH), lactate dehydrogenase (LDH), isocitrate dehydrogenase, succinate dehydrogenase, β-hydroxybutylate dehydrogenase (β-HBDH) and monoamine oxidase (MAO) in the cardiac muscle were determined histochemically. β-HBDH activity was greatly increased in the stage of developing hypertension in SHR. LDH activity increased simultaneously with the rise of β-HBDH activity. Moreover, MAO activity increased markedly in later stages when the blood pressure was already elevated in SHR. To confirm the histochemical findings of β-HBDH activity, the mitochondrial fraction of cardiac muscle was subjected to chemical assay. The chemical findings of myocardial β-HBDH in SHR corresponded well with the histochemical findings. The myocardial β-HBDH activity in SHR increased markedly at the age of 5 to 9 weeks, while no or minimal activity was found in controls of the same age. No significant difference of β-HBDH activity was observed between SHR and controls in the mitochondrial fraction from the diaphragm and liver. The increase of β-HBDH activity in the cardiac muscle of SHR prior to the development of cardiac hypertrophy suggests that the metabolism of ketone bodies may play an important role in providing the energy necessary for the development of cardiac hypertrophy in SHR.

Additional Indexing Words:
Cardiac metabolism β-hydroxybutyrate dehydrogenase Histochemistry Enzyme Hypertension

It is well known that the heart adapts to an over-load by hyperfunction and later hypertrophy of the myocardium.1,2 Much energy is necessary for the heart to make the compensatory changes, during which hypertrophy is the result of protein synthesis.2–4
Hypertrophy of the heart has been investigated intensively from many points of view, anatomic,5)-6) hemodynamic,7)-9) biochemical,10)-12) etc. Studies of the energetics of the heart might give some clue to the mechanism of cardiac hypertrophy as well as of heart failure.

Much research has been done on the biochemistry of cardiac hypertrophy. Specimens were obtained from the hearts of patients with cardiac hypertrophy by biopsy during cardiac surgery13),14) and at autopsy.15) In addition, cardiac hypertrophy was induced in experimental animals by aortic constriction,2),11) renal hypertension,16),17) excessive loading of the heart18) or hypoxia.19) In these animals hypertrophy did not always occur, however, because even hypertension, for instance, was not always induced by constriction of the renal artery. Therefore, the results were not consistent, and interpretation was sometimes difficult.

In the spontaneously hypertensive rats (SHR) bred by Okamoto and Aoki20),21) severe hypertension develops in 100%,22),23) and these animals would be expected to develop cardiac hypertrophy.24),25) Therefore, SHR are considered good material for the investigation of cardiac hypertrophy.

Moreover, the blood pressure rises gradually from the 4th week after birth to reach its highest level almost always in the 17th week. Since cardiac hypertrophy seems to be proportional to elevation of the blood pressure, the hypertrophy can be investigated in all stages throughout the development of hypertension.

In order to investigate the biochemical changes in the myocardium, the author determined the activities of various enzymes in the myocardium by histochemical methods at all stages, i.e. pre- to late hypertensive stages. The morphological appearance was also investigated by light- and electron microscopy in cooperation with the colleagues.26)

$\beta$-hydroxybutyrate dehydrogenase, which was highest in the prehypertensive stages, was determined quantitatively also by a chemical method.

**Materials and Methods**

One hundred and forty-one spontaneously hypertensive rats (SHR) of F$_{19-24}$ were used with 140 control Wistar rats, from the Animal Center Laboratory of Kyoto University Faculty of Medicine. Of these animals, 84 SHR (F$_{19-22}$) and 79 control rats were used for histochemical and morphological studies, and the others for the chemical assay of $\beta$-HBDH activity in the mitochondria. The ages of the experimental animals were classified according to number of weeks after birth, so that rats aged 1 to 7 days were considered to be 1 week old.

Histochemical and morphological studies were performed at 2, 3, 4, 5, 6, 7, 9, 11, 13, 15, 17, 19, 21, and 23 weeks, and 1 year after birth. Chemical assay was undertaken at 4, 5, 6, 7, 9, 13, and 23 weeks, and 1 year after birth.
Blood pressure and body weight: The rats were weighed weekly after weaning and just before decapitation, except that rats aged 2, 3, and 4 weeks were weighed only once prior to decapitation. The blood pressure was determined by tailwater plethysmography without anesthesia. Blood pressure was read 3 times and the average value was calculated.

Heart weight: The SHR and control rats of the same age were killed simultaneously by decapitation. The hearts were extirpated immediately and weighed after removal of both auricles. The ratio of heart weight to body weight was calculated and evaluated as an index of cardiac hypertrophy.

Histochemical method: The heart was cut across the middle between the apex and the base. The apical parts of the hearts from both SHR and controls were fixed together on a wooden block side by side. The preparations were frozen rapidly in n-hexan cooled with acetone and dry ice, and stored in a deep freezer at $-20^\circ$C. They were cut 12 $\mu$ thick by a Lipshaw cryostat (Model No. 1500) at $-20^\circ$C, mounted on cover-slips, and kept in a deep freezer again. The sections, after being left to dry at room temperature, were stained for the following enzymes: glucose-6-phosphate dehydrogenase (G6PDH), lactate dehydrogenase (LDH), $\beta$-hydroxybutyrate dehydrogenase ($\beta$-HBDH), succinate dehydrogenase (SDH), isocitrate dehydrogenase (IDH), and monoamine oxidase (MAO). The methods used for staining are listed in Table I. These stained sections were postfixed with 8% neutral formalin for about 50 min and mounted in Apathy gummy syrup.

Table I. Histochemical Methods of Demonstrating Various Enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Incubation period</th>
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<tbody>
<tr>
<td>G6PDH</td>
<td>Nachlas, Walker and Seligman$^{27}$</td>
</tr>
<tr>
<td>LDH</td>
<td>Nachlas, Walker and Seligman$^{28}$</td>
</tr>
<tr>
<td>IDH</td>
<td>Nachlas, Walker and Seligman$^{27}$</td>
</tr>
<tr>
<td>SDH</td>
<td>Nachlas, Tsou, De Souza, Cheng and Seligman$^{29}$</td>
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<tr>
<td>$\beta$-HBDH</td>
<td>Nachlas, Walker and Seligman$^{28}$</td>
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<tr>
<td>MAO</td>
<td>Glenner, Barthner and Brown$^{30}$</td>
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Histological method: The basal parts of the heart were fixed in 10% formalin, embedded in paraffin, cut 4 $\mu$ thick, stained with hematoxylin-eosin and Mallory’s azan for the morphological studies. The diameters of myocardial cells were measured on a cross section across the nucleus of the cells by the micrometer in the microscope. The mean value of the diameter of the cell was calculated from 100 cells for each heart.

Chemical determination of the activity of $\beta$-HBDH in the myocardium: The heart, after being weighed, was cut finely with scissors, and homogenized during cooling in ice water. The mitochondrial fraction, which was separated by a modification of Hagihara’s method$^{31}$ as depicted in Fig. 1, was mixed with 1 to 3 ml of 0.25 M sucrose solution. The content of protein in this mixed emulsion was determined by the method of Lowry et al.$^{32}$ In a modification of Sekuzu’s method,$^{33}$ 0.1 ml of this emulsion was added to the mixed solution of NAD and $\beta$-hydroxybutyrate in a cuvette with 1 cm light path. The change in optical density at the wave length of 340 mA was recorded automatically by a Shimazu spectrophotometer (Model No. MPS 50). The value of the optical density after 10 min...
was divided by the protein content of this emulsion, and the resultant value (optical density/mg of protein) was considered as the activity of $\beta$-HBDH.

Chemical determination of mitochondrial $\beta$-HBDH activity in the diaphragm: The mitochondrial fraction of the diaphragm resected from SHR and control rats, at the age of 5, 7, and 9 weeks and 1 year, was prepared and assayed for $\beta$-HBDH activity by the same method as that used for the myocardium.

Chemical determination of mitochondrial $\beta$-HBDH activity in the liver: The mitochondrial fraction was prepared from 1 Gm of liver, which was resected from the right lobe of SHR and control rats at the age of 5, 7, and 9 weeks and 1 year, and subjected to the determination for the activity of $\beta$-HBDH by the same method as that used for the myocardial tissue except that the recording time in spectrophotometry was limited to 3 min.

RESULTS

Blood pressure: The blood pressure in SHR rose to 140±5 mmHg at the age of 7 weeks and was significantly higher than that in control rats, which was always lower than 140 mmHg throughout the experimental period. It was over 150 mmHg, 180±5 mmHg and 195±10 mmHg at the age of 9 weeks, 17 weeks and 1 year, respectively. The blood pressure in male animals was higher by 5 to 10 mmHg than that in female in both SHR and
controls (Fig. 2).

Body weight: There was no significant difference in body weight between SHR and controls.

Heart weight: No significant difference in the heart weight was found between the 2 groups until the age of 9 weeks, and then the heart weight increased faster in SHR than in controls.

Ratio of heart weight to body weight: Until the age of 11 weeks, this ratio decreased in both groups in a similar trend. In SHR it started to rise after the age of 13 weeks, while in the controls it continued to decrease until the age of 15 weeks, and then remained constant. Consequently, there was a significant difference in the ratio after the age of 15 weeks between the 2 groups (Fig. 2).

Diameter of the myocardial cells: In both groups the diameter of the myocardial cells increased after the age of 2 weeks to 13.5±0.8 μ at the age
Table II. Activity of Enzymes in Myocardium of SHR

<table>
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<th>Enzyme</th>
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<th>19</th>
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<th>One year</th>
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<tbody>
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<td>G6PDH</td>
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<td>LDH</td>
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<td>β-HBDH</td>
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<td>SDH</td>
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Notes: – decreased, ± equivocal, + slightly increased, ++ moderately increased, +++ markedly increased, ++++ very markedly increased.

of 7 weeks. Thereafter, it increased rapidly in SHR, but not in the controls. It was 17.0±1.0 μ in SHR and 14.2±0.8 μ in the controls at the age of 13 weeks, when the difference was conspicuous between the 2 groups. Then it increased gradually with aging (Fig. 2).

Histochemical findings: The activity of the enzymes was evaluated semiquantitatively, by comparing the strength of staining and width of the stained area of the myocardial cells in SHR with those in controls; slightly decreased (−), equivocal (+), slightly increased (+), moderately increased (++), markedly increased (+++), and very markedly increased (++++) (Table II). G6PDH: Although the activity of this enzyme was generally low in both SHR and controls, a slight increase was seen in SHR at the age of 7 to 9 weeks and 15 to 19 weeks. LDH: In SHR a slight increase in the activity of this enzyme was observed after the age of 4 weeks until the age of 19 weeks, after which no difference was seen between the 2 groups. SDH: The activity of this enzyme was increased at the age of 5 to 19 weeks. IDH: a slight decrease in the activity of IDH was seen in SHR at the age of 5 to 11 weeks, whereas no significant difference was found between SHR and controls at other ages. β-HBDH (Fig. 3 a–f): At the age of 4 weeks the activity of β-HBDH in the myocardium, i.e. the intensity of staining showed no differences between SHR and controls. However, fine granules stained in deep violet microscopically appeared in the myocardial cells in the ventricular septum of SHR at the age of 4 weeks, while in the controls such cells were first observed at the age of 6 weeks. After the age of 9 weeks the granules in the cells were increased markedly in number to be confluent in SHR, whereas they were found to increase slightly, but not to appear in confluence in the controls. Therefore, there were significant differences between SHR and controls. Thereafter, the intensity of staining remained steady in SHR,
Fig. 3. Histochemistry of β-HBDH of the myocardium in SHR (right) and control (left).

a) At the age of 4 weeks. Slightly increased β-HBDH activity localized in the septal region of left ventricle in SHR.

b) At the age of 5 weeks. Markedly increased β-HBDH activity in SHR.

c) At the age of 7 weeks. Markedly increased β-HBDH activity in SHR.

d) At the age of 9 weeks. Markedly increased β-HBDH activity in SHR and slightly increased activity in control.

e) At the age of 13 weeks. Markedly increased β-HBDH activity in SHR and moderately increased activity in control.

f) At the age of 23 weeks. No significant difference of β-HBDH activity between SHR and control.
while it increased gradually in the controls. And so at the age of 13 weeks there was only a slight difference, which was no longer observed at the age of 23 weeks. MAO: In both groups no MAO activity was found until the age of 9 weeks, when in SHR the activity appeared first in the myocardial cells, especially in perivascular regions. Thereafter, it was high from the 11th week in SHR, while the increase was slight and only after the age of 15 weeks in the controls. Thus, there were significant differences between the 2 groups.

Quantitative observation of the mitochondrial $\beta$-HBDH activity by chemical method: As seen in Fig. 4, no $\beta$-HBDH activity was observed in the mitochondrial fraction of cardiac muscle of SHR or controls at the age of 4 weeks. In SHR the activity had already increased by the age of 5 weeks and reached its peak (0.038±0.015 O.D./10 min/mg protein) at the age of 7 weeks. On the other hand, in the controls it started to increase at the age of 9 weeks, amounting to 0.016±0.003 O.D./10 min/mg protein at the age of 13 weeks, when no significant difference was observed between SHR and

![Fig. 4. Mitochondrial $\beta$-HBDH activity (chemical method) in heart, diaphragm and liver.](image-url)
controls. At the age of 1 year $\beta$-HBDH activity was lower in SHR than in the controls.

In the diaphragm and liver, no significant differences were found between SHR and controls.

**DISCUSSION**

In many cardiac diseases such as valvular disease, hypertensive heart disease, arteriosclerotic heart disease, congenital heart disease, enlargement of the heart is observed clinically; dilatation and/or hypertrophy of the heart. When an overload to the heart is applied in these diseases, the heart seems at first to adapt to the load with an increase in cardiac output due to tachycardia and dilatation of the heart, according to Starling's law; an increase in the end-diastolic volume of the ventricle produces an increase in stroke volume. However, if the volume is increased beyond a certain point, the stroke volume is decreased. Then the heart begins to adapt to the load by hypertrophy. Although it used to be thought that the development of hypertrophy was gradual, it was proved by Fanburg, Schreiber et al and others in their biochemical studies on DNA and RNA that hypertrophy begins within a few hours after the overload.

Morphologically, hypertrophy of the heart means an increase in thickness of the ventricular wall, due to an increase in the diameter of the myocardial cells, accompanied on occasion by proliferation of connective tissue. There is, moreover, the problem of hyperplasia, i.e. an increase in the number of myocardial cells, as discussed by Linzbach. All these phenomena require the synthesis of protein, for which much energy is needed.

There have been many studies on the hypertrophy of the heart from various points of view, morphologic, hemodynamic, biochemical, etc. This paper describes an investigation of the mechanism of the development of cardiac hypertrophy in experimental animals from the aspect of energetics determining the activity of enzymes in the myocardium by histochemical procedures and by chemical methods.

In SHR used in this study, the blood pressure rose gradually from the age of 7 weeks, and reached to $180\pm5$ mmHg at the age of 23 weeks. It was $195\pm10$ mmHg after 1 year. The ratio of the heart weight to body weight increased gradually from the age of 11 weeks to 23 weeks, although it had decreased from the age of 3 weeks to 11 weeks. The diameter of the myocardial cells increased gradually from the age of 3 weeks to 23 weeks and even for 1 year. These results indicate that hypertrophy of the heart develops gradually almost in parallel with the elevation of blood pressure,
as expected.

As mentioned above, a great deal of energy is needed for the development of cardiac hypertrophy throughout the whole period, during which there is not only hyperfunction of the heart but also protein synthesis. In general, there must be production and conservation of energy before its utilization. Substrates and enzymes are needed for the former.\textsuperscript{38)}

The substrates utilized in the heart are considered to be glucose, fatty acids, lactate, ketone bodies, etc, which are probably derived from the blood. Many investigations have been conducted in human subjects and experimental animals with respect to the main substrates in the heart. In their studies on coronary sinus blood in human subjects, Bing et al,\textsuperscript{39)} Gordon et al,\textsuperscript{40)} and others\textsuperscript{41)} have demonstrated that the myocardial extraction ratio of fatty acids was particularly great after a high fat intake, and that fatty acids were used as a major source of energy in the myocardium. However, Bing et al\textsuperscript{42)} showed that carbohydrate, i.e. glucose, lactate, and pyruvate, was utilized as a main source of myocardial energy after glucose infusion. In experimental animals Brachfeld et al,\textsuperscript{43)} Morgan et al,\textsuperscript{44)} and others\textsuperscript{45)} also emphasized an increase in carbohydrate utilization in the cardiac muscle, while Evans,\textsuperscript{46)} Shipp,\textsuperscript{47)} and others\textsuperscript{48)} considered that lipid was a major source of energy in the myocardium. Moreover, Williamson et al,\textsuperscript{49)} Bassenge et al,\textsuperscript{50)} and others\textsuperscript{51)} noted that the isolated perfused rat heart utilized ketone bodies in preference to glucose and other endogenous substrates.

In energy production from substrate, the Embden Meyerhof pathway, \(\beta\)-oxidation of fatty acid, citric acid cycle, etc must participate, so many corresponding enzymes should be active. Among them G6PDH and LDH were chosen in this study as enzymes involved in carbohydrate metabolism, \(\beta\)-HBDH in the metabolism of ketone bodies, and IDH and SDH in the citric acid cycle.

In the present histochemical investigation of the activity of enzymes in the myocardium of SHR, LDH and SDH showed an increase from the age of 4 or 5 to 19 weeks and G6PDH a slight increase from 7 to 9 weeks and 15 to 19 weeks, while IDH decreased from 5 to 11 weeks, as shown in Table II. In regard to \(\beta\)-HBDH, it was quite different from other enzymes in that its activity showed a remarkable increase from the age of 5 to 11 weeks, i.e. in the stage of gradual elevation of blood pressure. Moreover, there was also a marked increase in the activity of MAO in later stages when the blood pressure was already elevated.

In regard to the activity of MAO in the myocardium, Tabei\textsuperscript{52)} demonstrated almost the same results in SHR, and de Champlain et al\textsuperscript{16)} also in rats with Goldblatt’s hypertension, DOCA hypertension, and salt hyper-
tension. In these investigations the increase in MAO activity was found in the stage of developed hypertension, in which catecholamines are assumed to play a role.

Ito\textsuperscript{53} and Shipp et al\textsuperscript{54} showed by using \textsuperscript{14}C-labeled glucose that the pentose shunt was either quiescent or only moderately activated in normal heart tissue. However, the shunt increased its activity in the myocardium of the following: In the early stage of renal hypertension in rats,\textsuperscript{55} in chick embryos,\textsuperscript{56} and after myocardial infarction in dogs.\textsuperscript{57} In the former 2 the heart muscle might develop hypertrophy and/or hyperplasia and in the latter reparative processes, all of which should be induced by synthesis of protein. In regard to the activity of G6PDH, a key enzyme in the pentose shunt, Valadaves et al\textsuperscript{58} also demonstrated that it increased in the myocardium of rats with Goldblatt’s hypertension. Nagano and his coworkers\textsuperscript{17} observed an increase in the activity of G6PDH, glycer-aldehyde-3-phosphate dehydrogenase and LHD in the myocardium, whereas the activity of phosphofructokinase, IDH and malate dehydrogenase showed a decrease in rats with hypertension induced by Goldblatt’s procedure and by Masugi-nephritis. They suggested an impairment of oxidative breakdown and an increase in anaerobic glycolysis through the pentose shunt.

Concerning the cardiac metabolism in SHR, Ooshima\textsuperscript{59} showed that the activity of LDH was increased at the age of 3 to 6 months, while that of G6PDH, alkaline phosphatase, and acid phosphatase was obscure at all stages of life.

In the present study, the activity of G6PDH increased slightly in the stage of developing hypertension. This finding might show hyperfunction of the pentose shunt in carbohydrate metabolism. The activity of LDH in the present study also showed an increase during the stage of developing hypertension. It is considered to be due to hyperfunction of carbohydrate metabolism.

In regard to the enzymes related to the citric acid cycle, Nagano et al\textsuperscript{17,60} reported that IDH and MDH activity decreased in cardiac hypertrophy induced by renal hypertension as well as in acute cardiac failure produced by aortic constriction in rats. In the present investigation SDH and IDH in the myocardium of SHR showed different results in the stage of developing hypertension, where the activity of the former was slightly increased and that of the latter slightly decreased. It is very difficult to interpret these results. Although the citric acid cycle in intact tissue and isolated mitochondria was considered to be controlled at the levels of citrate synthetase, IDH (NAD-linked) and \(\alpha\)-ketoglutarate dehydrogenase,\textsuperscript{61} the regulation seemed to depend upon the quality and quantity of substrates in the citric acid cycle.
Therefore, the activity of some enzymes related to the substrates might be increased, and that of others decreased.

Histochemical observations showed that $\beta$-HBDH activity in the myocardium was markedly increased in the stage of developing hypertension in SHR. As the histochemical assay is semiquantitative, a chemical method was also used to confirm the increase in $\beta$-HBDH activity. As shown in Fig. 4, the results of the chemical study of myocardial $\beta$-HBDH activity in SHR corresponded well with those of the histochemical study. $\beta$-HBDH activity in the myocardium increased rapidly starting 5 weeks after birth and reached its peak (0.038±0.015 O.D./10 min/mg protein) at the age of 7 weeks in SHR, while no or minimal activity was seen in controls of the same age. However, in regard to $\beta$-HBDH activity in the diaphragm and liver, no significant difference was observed between SHR and controls during the entire period (Fig. 4). Therefore, it seemed likely that the rise of $\beta$-HBDH activity observed only in the myocardium has an important meaning.

Although there is no evidence that ketone bodies are major substrates of the human heart except in conditions such as starvation or diabetes mellitus with severe ketoacidosis, it is known that ketone bodies are usually present in high concentrations to be utilized as important substrates in experimental animals such as rats and dogs. Bassenge and co-workers demonstrated in intact anesthetized dogs that the administration of acetoacetate caused a marked depression in the myocardial utilization of free fatty acid and pyruvate and a marked increase in the myocardial extraction of lactate.

In the present study the activity of LDH increased simultaneously with the rise of $\beta$-HBDH activity. This suggests that ketone bodies may be utilized in preference to glucose in the early stage of hypertrophic myocardium in SHR.

As genetic factors are considered to play an important role in the hypertension of SHR, the increase in $\beta$-HBDH activity also might be controlled by strain specific factors. It was, however, found only in the myocardium, and not in the diaphragm or liver. Therefore, it is feasible to consider that this change in $\beta$-HBDH was more closely related to the development of cardiac hypertrophy than to genetic factors.

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