Vascular Protein Metabolism in the Pathogenesis of Hypertension

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Labelled proline incorporation into collagenous and noncollagenous proteins of aorta or mesenteric arteries was significantly increased in 70-day-old spontaneously hypertensive rats (SHR) at the early stage of hypertension in comparison with normotensive Wistar-Kyoto (WK) rats; however such an increase was not detected in 30-day-old SHR at the prehypertensive stage. Similar increases in the proline incorporation were noted in 70-day-old renal hypertensive rats and in DOCA hypertensive rats in which hypertension had been induced similarly to that in SHR. Furthermore, the decay of the specific activity of noncollagenous and collagenous proteins was studied for 100 days after labelled proline infusion. The decay of the noncollagenous protein activity was clearly accelerated in the heart, aorta and especially in the mesenteric arteries of SHR compared with WK. The decrease in the hydroxyproline radioactivity of the collagenous protein was significantly faster in the aorta and mesenteric arteries in SHR. These results proved the increased protein metabolism in the arterial walls in the relatively early stage of hypertension in SHR as well as in experimental hypertension, and then suggested its improtance in the common pathogenetic mechanisms of hypertension.

SPONTANEOUSLY hypertensive rats (SHR)¹,² are now used all over the world and regarded as the best animal models so far for essential hypertension in man³,⁴ The pathogenesis of hypertension in SHR, although not yet completely clarified as that in essential hyper-

tension, has been intensively studied. The importance of neurogenic factors was indicated by the destruction of the central nervous system⁵ the recording of sympathetic discharge⁶ the hind-limb perfusion experiment⁷ and noradrenaline turnover studies⁸. However, neurogenic components seemed to retreat after the establishment of hypertension, because the increased noradrenaline turnover in the heart⁹ and high plasma dopamine β-hydroxylase level⁵,⁹ were clearly detected only in the pre-hypertensive or early hypertensive stages.

On the other hand, nonneurogenic increase in peripheral vascular resistance was proven by various perfusion experiments⁶,¹⁰ and structural vascular alterations were histometrically con-

Key Words:
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firmed at the relatively early stage of hypertension.\textsuperscript{1,12}

In order to clarify the intervening mechanism between the initial neurogenic components and subsequent structural vascular alterations, proline incorporation into the collagenous and noncollagenous proteins of arterial walls\textsuperscript{7} were studied in SHR as well as in other experimental hypertensive rats. The decay of the radioactivity from arterial walls after proline incorporation was also observed to confirm the findings of the incorporation studies.

\textbf{MATERIALS AND METHODS}

SHR (\textsuperscript{F_{31-33}} : stroke-resistant substrains) and Wistar-Kyoto rats, from which SHR was derived, were chosen for the experiments from the original colony maintained at the Department of Pathology, Faculty of Medicine, Kyoto University. Renal infarction hypertension\textsuperscript{13} were induced by the ligation of bilateral posterior branches of renal arteries, and DOCA hypertension\textsuperscript{14} was made by the implantation of 25 mg of DOCA pellet into the psoas muscle in 40-day-old WK. After these operations, the rats were given 1% saline for drinking water. Blood pressure was checked once weekly by an indirect tail-pulse-pick-up method.\textsuperscript{15} They were all kept under a constant standard laboratory condition.\textsuperscript{2,3}

In a preliminary experiment, \textsuperscript{3}H-proline (1 \textmu Ci/g of L-proline \textsuperscript{3}H(G), 63 Ci/mmole, Daiichi Pure Chemical Co.) was injected intravenously in 12 SHR and 12 WK. Then 3 of each group were sacrificed 2, 4, 10 and 24 hours after the injection to determine the time course of \textsuperscript{3}H-proline incorporation into collagenous and noncollagenous proteins of the aorta and mesenteric arteries.

In the first series of the experiment, 10 SHR and 12 WK at the age of 30 days, and also 10 SHR, 10 WK, 5 renal hypertensive rats and 10 DOCA hypertensive rats at the age of 70 days were starved overnight. They were then sacrificed by decapitation 4 hours after the intravenous injection of \textsuperscript{3}H-proline (0.7 \textmu Ci/g), to determine the incorporated radioactive proline in the aorta and mesenteric arteries.

In the second series of the experiment, \textsuperscript{14}C-proline (0.25 \textmu Ci/g of proline \textsuperscript{14}C(U), over 225 mCi/mmmole, New England Nuclear) was intravenously injected in 15 SHR and 15 WK at the age of 42 days. Then five rats of each group were sacrificed by decapitation 30, 60 and 100 days after the \textsuperscript{14}C-proline injection, to determine the radioactivity in the collagenous and noncollagenous proteins of the heart, aorta and mesenteric arteries.

When these animals were sacrificed, blood was collected to determine serum radioactivity. Also, the heart, aorta (the ascending and descending aorta down to the right renal artery), and superior mesenteric arteries from the root to the branches leading to the intestinal loop were immediately extirpated. After blood was washed out of the vasculature with physiological saline,
fat and loose connective tissue around the aorta and mesenteric arteries were carefully removed with small forceps in physiological saline. The tissues were blotted on filter paper for checking the wet weights.

The principle for extracting collagenous and noncollagenous proteins was shown in Fig. 1. After the tissues were homogenized in 5% trichloroacetic acid (TCA), collagen was extracted with 5% hot TCA at 90°C for 30 minutes by the method of Fitch et al. The extraction was repeated and the precipitate was washed twice with cold 5% TCA. Combined TCA extracts were mixed with an equal volume of concentrated HCl and then hydrolyzed at 120°C for 17 hours in an autoclave. Hydroxyproline in the hydrolysate was determined after Prockop and Udenfriend; the radioactivities of the toluene extract for hydroxyproline assay were counted in toluene scintillation solution by a liquid scintillation spectrometer (Packard, model 2002) to determine the specific activities of hydroxyproline.

The precipitate of noncollagenous protein were homogenized in 0.1 N NaOH and kept overnight at 4°C in a refrigerator. After centrifugation the soluble, noncollagenous protein was mixed with one-tenth of the volume of 50% TCA and chilled in ice for 30 minutes. The precipitates obtained after centrifugation were washed 3 times with 5% TCA, then heated in 5% TCA at 85°C for 30 minutes. The noncollagenous protein preparations thus obtained were washed twice with acetone and then with ethanol. Finally they were dissolved in formic acid for the determination of radioactivities in BBOT scintillation solution and for the protein assay. The specific radioactivities per mg noncollagenous protein were calculated.

To determine the initial serum radioactivity, small blood samples (50 μl) were also collected into hematocrit capillary tubes through a cut at the tip of the tails 5 minutes after the proline infusion in the first experiment. Total free proline in the plasma obtained by decapitation was measured by the photometric method of Troll and Lindsley.

Mean ± standard error of the mean (SE) of all numerical data was calculated and the statistical differences between groups were checked by Student’s t test.

RESULTS

A preliminary experiment showed the time course of the 3H-proline incorporation into the aorta and mesenteric arteries in SHR and WK (Fig. 2). The incorporation into both collagenous and noncollagenous proteins 4 hours after the injection was nearly the peak value and showed almost similar patterns to those indicated.

Fig. 2. Hydroxyproline radioactivity incorporated into the vascular collagenous protein after 3H-proline injection in SHR and Wistar-Kyoto.
TABLE I 3H-PROLINE INCORPORATION INTO VASCULATURES IN SHR, RENAL AND DOCA HYPERTENSIVE RATS

<table>
<thead>
<tr>
<th>Age</th>
<th>Experimental groups (No. of Rats)</th>
<th>Blood Pressure (mmHg)</th>
<th>Collagenous Protein (dpm/µ moles hyp.)</th>
<th>Noncollagenous Protein (dpm/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30* or 40 days old</td>
<td>Aorta</td>
<td>Mesenteric Artery</td>
</tr>
<tr>
<td>30 days old</td>
<td>SHR (10)</td>
<td>121 ± 5*</td>
<td>4563 ± 633</td>
<td>3387 ± 540</td>
</tr>
<tr>
<td></td>
<td>Wistar-Kyoto (12)</td>
<td>109 ± 4*</td>
<td>4638 ± 678</td>
<td>3789 ± 336</td>
</tr>
<tr>
<td>70 days old</td>
<td>SHR (10)</td>
<td>138 ± 5</td>
<td>2030 ± 129**</td>
<td>1446 ± 109**</td>
</tr>
<tr>
<td></td>
<td>Wistar-Kyoto (10)</td>
<td>134 ± 4</td>
<td>1416 ± 105</td>
<td>916 ± 58</td>
</tr>
<tr>
<td></td>
<td>Renal hypertension (3)</td>
<td>129 ± 5</td>
<td>1421 ± 111</td>
<td>1414 ± 100†</td>
</tr>
<tr>
<td></td>
<td>DOCA hypertension (10)</td>
<td>126 ± 6</td>
<td>181 ± 3***</td>
<td>1504 ± 100</td>
</tr>
</tbody>
</table>

Mean ± SE  
++ Significant differences from age-matched Wistar-Kyoto 
++++ hyp: hydroxyproline 
Rats, sacrificed 4 hours after 3H-proline injection (0.7μC/g, i.v.)

\[ p < 0.05 
\[ p < 0.01 
\[ p < 0.001

In Fig. 2. Thus, the time period of 4 hours appeared to be optimal, and it was chosen for the following experiment. Although the number of rats was limited, the incorporation of 3H-proline into both protein fractions was greater in SHR than in WK.

In the first series of the experiment (Table I) blood pressure in 30-day-old SHR at the prehypertensive stage was not so different from that in WK. The proline incorporations into the collagenous protein in the aorta and mesenteric arteries were not different between these two groups, but proline incorporation into the noncollagenous protein was significantly increased in the aorta of SHR. Blood pressure in 70-day-old SHR was significantly higher than in age-matched WK, but not significantly different from that in renal or DOCA hypertensive rats. The proline incorporation into the collagenous protein of the mesenteric arteries was significantly increased in all hypertensive groups, as compared with WK. The proline incorporation into the collagenous protein of the aorta was significantly increased only in SHR. On the other hand, proline incorporation into the noncollagenous protein of mesenteric arteries was increased in all hypertensive groups, and significantly so in SHR and DOCA hypertensive rats. The incorporated radioactivity of noncollagenous protein in the aorta was significantly higher in SHR and renal hypertensive rats.

There was no difference in plasma proline level between SHR (35 ± 3 µg/ml) and WK (40 ± 4) at the age of 30 days, and also no difference in this level among DOC (29 ± 3), renal hypertensive rats (31 ± 4), SHR (38 ± 3) and WK (37 ± 5) at the age of 70 days. Decline of plasma radioactivity for 4 hours after the 3H-proline injection showed no significant difference between SHR and WK, or among various hypertensive rats.

In the second series of the experiment the decay of the specific activity of noncollagenous and collagenous proteins was observed in SHR and WK for 100 days after the 14C-proline infusion. The results are plotted in Fig. 3. The decay of the noncollagenous protein activity was clearly accelerated in the heart, aorta, and especially in the mesenteric arteries of SHR, as compared with those in WK. The decrease in the

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The present results of the labelled proline incorporation study revealed that the incorporation into both collagenous and noncollagenous proteins of the aorta and mesenteric arteries was clearly increased in 70-day-old SHR at the early stage of hypertension. These results confirmed the preliminary report. However, such an increase was not proven in 30-day-old SHR, although the proline incorporation into the noncollagenous vascular protein tended to be increased. Similar increases in the proline incorporation into the vascular walls were noted especially in mesenteric arteries in renal and DOCA hypertensions one month after the onset of hypertension. As prolonged antihypertensive therapy suppressed such an enhanced incorporation in SHR metabolic enhancement of vascular thickening, especially the increased incorporation into collagenous protein, appeared to be secondary to hypertension. In this experiment, neither free proline level nor decline in radioactive proline in the plasma was significantly different among various hypertensive and normotensive groups. Although the intracellular level of radioactive proline was not determined, the increased incorporation of proline was presumed to correspond to the increased protein synthesis. This was presumed because similar increases in $^3$H-proline incorporation into the collagenous protein of mesenteric arteries were also observed in "in vitro" experiments (Yamori et al., unpublished data). Futhermore, Ooshima et al. examined various indices of vascular collagen synthesis in SHR and DOCA hypertension. They proved a clear increase in prolylhydroxylase activity in SHR even 50 days after birth and in DOCA hypertension. The present study added a new finding: the decay of hydroxyproline specific activity of the collagenous protein in the aorta and mesenteric arteries was faster in SHR than in WK. All these findings indicate that hypertension itself enhances vascular collagen synthesis in relatively short duration, and it accelerates arteriosclerosis.

On the other hand, hypertension seemed to enhance not only vascular collagen synthesis but also noncollagenous protein metabolism secondarily ("adaptive metabolic change of vascular walls"). This was indicated by the increased proline incorporation into the noncollagenous protein in the arterial wall, especially into such a muscular type artery as the me-
senteric artery in various hypertensive rats. The accelerated decay of the specific radioactivity of noncollagenous protein was clearly observed in the aorta, mesenteric artery and the heart of SHR, as compared with WK. This finding further supported the increased noncollagenous protein metabolism in the cardiovascular system under hypertensive state. Such an enhancement of vascular noncollagenous protein metabolism is thought to be mainly secondary to hypertension, because a similar finding was also noted in other experimental hypertensions. However, a significant increase in labelled lysine incorporation into the noncollagenous protein of mesenteric arteries was noted in SHR even in the prehypertensive stage by Yamabe and Lovenberg. The decay in the specific activity of noncollagenous protein, as observed by the present study, strongly suggested that the initial proline incorporation into the mesenteric arteries was already increased in SHR at the age of 42 days, as indicated by the y-intercept of the extended line of the decay. Our recent study further offered a new finding that the increased lysine incorporation into the noncollagenous protein of mesenteric arteries in SHR was attenuated by sympathetic denervation. This study revealed a close relationship between sympathetic innervation and protein metabolism in the arterial wall. It further indicated its pathogenetic importance in the development of hypertension ("neuro-vascular linkage" in hypertension).

These findings, together with various results obtained up to the present, seem to throw new light on the pathogenesis of spontaneous hypertension. In the initiation mechanism of hypertension, the peripheral vascular resistance increases neurogenically to cause labile hypertension. While increased vasmotor tone itself enhances noncollagenous protein metabolism ("neuro-vascular linkage"), the increased pressure load to vascular walls due to hypertension accelerates noncollagenous as well as collagenous protein metabolisms ("adaptive metabolic change"). The increased noncollagenous protein metabolism seems to lead to medial hypertrophy, i.e., "adaptive structural vascular change", the pathogenetic importance of which has been pointed out by Folkow et al. in spontaneous and essential hypertension. On the other hand, the increased collagenous protein metabolism results in hypertensive diffuse atherosclerosis. These metabolic vascular alterations are thought to be mainly a biochemical adap-

tation of the arterial walls to high pressure load "adaptive metabolic change" and to precede "adaptive structural change". Both of these adaptive metabolic and structural changes are secondarily involved in increasing the peripheral vascular resistance, i.e., the maintenance mechanism of hypertension, and they seem to be the final common mechanisms of hypertension, whatever the primary cause may be. Through the metabolic vascular changes any primary causes of hypertension are subsequently substituted by the final common mechanism, and these causes gradually become obscure. This is likely the main reason why the labile hypertension is gradually fixed, or why the primary cause of essential hypertension remains obscure up to the present.

Acknowledgement

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REFERENCES


Discussion

Chairman: NOBUHIKO SHIBATA, The Center for Adult Diseases, Osaka

Dr. SHIBATA: In this paper, the following problems were discussed.

1) What kind of protein was involved in the so-called noncollagenous proteins and how about the metabolism of elastin and smooth muscular fibers in arterial wall? It might be considered that elastin and the muscular fibers were mainly located at arterial media and likely participated in induction of the initial labile hypertensive state through the elasticity or contractility. (Dr. KOKUBU, Ehime Univ.)

2) Which factor (neurogenic or hypertensive) was more essential for acceleration in the metabolism of arterial noncollagenous proteins at the labile hypertensive stage? (Dr. KANEKO, Yokohama City Univ.)

3) Did the regional arteriosclerotic lesion with enhanced collagen synthesis play a role as an etiological factor for hypertension? (Dr. KANEKO)

Dr. YAMORI's Comment:

1) Noncollagenous proteins contained alkaline soluble proteins except elastin, which were mainly located at arterial media. The important point in this paper consisted in the fact that metabolism of noncollagenous proteins in arterial wall had been already accelerated even at the very early stage of hypertension (labile hypertensive stage). In this stage, no morphological
alteration could be detected in the arterial wall. In other words, the acceleration of the arterial protein metabolism seemed not to be initiated prior to hypertensive state, but it followed after initiation of high blood pressure.

2) The acceleration of noncollagenous protein metabolism in mesenteric arteries was attenuated by surgical or pharmacological sympathetic denervation. On the other hand, secondary acceleration of the protein metabolism followed by high blood pressure was also found in other experimental hypertensive animals than SHR. From these findings, it was reasonable to expect that the accelerated metabolism of arterial noncollagenous proteins found at the very early stage of SHR was induced not only by hypertension, but also by neurogenic factor.

In the case treated with hydralazine, the arterial protein metabolism was accelerated with regardless of blood pressure. In the parallel experiment the drug was found to augment neural imput reflectively so the acceleration of protein metabolism due to the administration might be thought as a result of neural stimulation.

3) It is difficult to consider that regional arteriosclerosis did play a role as an etiological factor for hypertension. When synthesis of collagen in arteries was accelerated diffusely, the peripheral vascular resistance was considered to be enhanced, resulting in the elevation of blood pressure.

Dr. AOKI (Nagoya City Univ.): SHR in 30–40 days old was thought to be at the elevating stage and the one in 15 weeks old at the stable stage of hypertension. It is likely to assume that some regulating mechanisms by which further elevation of the pressure was inhibited were in operation in 15 weeks old SHR. Since, if the enhancement of the protein metabolism detected at both the elevating and stable stage caused to increase in vascular resistance it seems very difficult to keep the pressure at stable level in 15 weeks old SHR.