CHANGES IN VASCULAR WALL PRODUCTION OF PROSTACYCLIN AND THROMBOXANE A₂ IN SPONTANEOUSLY HYPERTENSIVE RATS DURING MATURATION AND THE CONCOMITANT DEVELOPMENT OF HYPERTENSION

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The purpose of this study was to clarify how the metabolism of vascular prostacyclin (PGI₂) and thromboxane (TX) A₂ in spontaneously hypertensive rats (SHR) is involved in aging and development of hypertension. We removed the aortic walls from 5-week-old and 20 to 25-week-old SHR and age-matched Wistar Kyoto rats (WKY). At 5 weeks of age, there was no significant difference in basal and maximal (arachidonic acid 0.1 mM) 6-keto-PGF₁₀α production between SHR and WKY, but the TXB₂ generation in the SHR aortic wall was markedly enhanced as compared with that in WKY. At 20 to 25 weeks of age, the SHR aortic wall synthesized about 1.5 times more 6-keto-PGF₁₀α in the basal condition and twice as much as in the maximal condition as did the WKY wall. However, there was no significant difference in TXB₂ production between SHR and WKY. Age-dependent increase of vascular 6-keto-PGF₁₀α was greater in SHR than in WKY. Moreover, the maximal/basal 6-keto-PGF₁₀α production ratio increased with age in SHR, but not in WKY. The synthesis of vascular TXB₂ was enhanced with age in WKY, but did not change with age in SHR. These data suggest that not only the enhanced basal generation of vascular 6-keto PGF₁₀α but also a much greater reservoir of 6-keto-PGF₁₀α synthesis in SHR was induced by both hypertension and maturity. The increased production of vascular TXB₂ in young SHR may affect the development of hypertension.

Prostacyclin (PGI₂), the major product of arachidonic acid (AA) metabolism in the vascular wall, has been reported to decrease vascular tonus directly and to participate in the regulation of blood pressure. Previous observations have provided the evidence for an enhanced synthesis of vascular PGI₂ in spontaneously hypertensive rats (SHR). Pace-Ascakis et al⁴ have reported an enhanced aortic synthesis of PGI₂ and increased conversion of AA to PGI₂ with age in SHR, and Botha et al⁵ and Uehara et al⁶ have shown that generation of vascular PGI₂ increases

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in mature SHR as compared with age-matched Wistar Kyoto rats (WKY). Moreover, Lukacsko\textsuperscript{4} has provided the evidence that an aortic synthesis of PGI\textsubscript{2} gradually increased with age in both SHR and WKY, and that aortic strips from 20-week-old SHR synthesized more PGI\textsubscript{2} than vessels from age-matched WKY. However, it is still unclear whether or not there is a close relation between PGI\textsubscript{2} synthesis and elevation of blood pressure.

Recently, it has been reported that thromboxane A\textsubscript{2} (TXA\textsubscript{2}), a potent platelet aggregating agent and vasoconstrictor, is also produced in the vascular tissue of rats\textsuperscript{5} and humans\textsuperscript{6}. This evidence indicates that the vascular TXA\textsubscript{2} production in SHR may increase arterial pressure. However, there have been few studies on the ability of aortic vessels to produce PGI\textsubscript{2} and TXA\textsubscript{2} in young and adult SHR in the presence or absence of various concentrations of exogenous AA.

The purpose of the present study is to clarify the metabolism of vascular eicosanoids in SHR from the viewpoints of aging and development of hypertension.

**MATERIALS AND METHODS**

1) Preparation of the aortic wall of SHR and WKY

Male 4-week -old SHR and age-matched WKY were purchased from Charles River Laboratories. They were allowed to acclimatize themselves to our laboratory for one week following arrival, because eicosanoid synthesis can be influenced by new environment. The rats were fed with regular rat diet and tap water ad libitum. Five-week-old SHR (n = 5) and age-matched WKY (n = 5), and 20 to 25-week-old SHR (n = 9) and age-matched WKY (n = 7) were used for the observation of eicosanoid synthesis in the aortic wall. Aortas were exposed through an abdominal incision under ether anesthesia, and perfused with ice-cold saline solution until the color of the flowing saline became completely clear in order to prevent the platelet attachment. Then the
Changes in Vascular Wall Production of Eicosanoids in SHR

Thoracic and abdominal aortas were rapidly removed, rinsed in ice-cold tris HCl buffer (50 mM, pH 7.5) and cleaned of adhering tissues. The cleaned aortas were cut open longitudinally, then divided into 4 strips (about 5 mm square, 10 mg) in the 5-week-old animals and 8 strips in the 20 to 25-week-old animals. In a preliminary investigation, no difference was found in eicosanoid synthesis between thoracic and abdominal aortas. In 5-week-old animals, one aortic strip was incubated in 1 ml of tris HCl buffer (50 mM, pH 7.5) without AA. Three additional strips were incubated at 37°C in 1 ml of tris HCl buffer with AA at the following final concentrations: 0.05, 0.1 and 0.3 mM. In 20 to 25-week-old animals, one was incubated without AA and the remainder were incubated with AA at the following final concentrations: 0.05, 0.1, 0.3, 0.6, 1.0, 5.0 and 10.0 mM. Incubation time was placed at 45 min, because the amounts of eicosanoid synthesized by aortic strips in SHR (n = 2) and WKY (n = 2) reached peak at 45 or 30 minutes after the initiation of incubation, as shown in Fig. 1 and 2. Aliquots after the incubation were stored at −80°C until the subsequent measurement of 6-keto-PGF₁α, a stable metabolite of PGI₂, and TXB₂, a stable metabolite of TXA₂.

Fig. 3. 6-keto-PGF₁α production in the aorta of 5-week-old SHR and WKY with and without the addition of AA. The aortic wall was incubated in tris HCl buffer (pH 7.5, 50 mM) for 45 min with and without the addition of AA. No difference in 6-keto-PGF₁α formation in the aorta between 5-week-old SHR and WKY was observed. The amount of 6-keto-PGF₁α produced by aortic wall increased significantly on adding AA.

Fig. 4. TXB₂ production in the aorta of 5-week-old SHR and WKY with and without the addition of AA. The amount of TXB₂ produced by aortic wall was significantly increased in SHR compared with that of WKY in all concentrations of exogenous AA. The synthesis of vascular TXB₂ was not enhanced by the addition of exogenous AA.

2) Measurement of blood pressure in rats
Systolic blood pressures in all conscious rats were measured the day before sacrifice by the tail cuff method using a programmed electrophygmonanometer PE-300 (Narco, Bio-System, Inc., Houston, Texas) after sedation of at least 15 min.

3) Radioimmunoassay of eicosanoids
Extraction of eicosanoids was performed by Inagawa’s method. Crude lipid extracts were obtained from samples (0.3 ml) by the procedure of Folch. Nonlipid contaminations were
removed on Sephadex G-25 columns. After evaporation of the eluted solution, the residue was dissolved in carbon tetrachloride and water was added. The aqueous phase was acidified with hydrochloric acid to pH 3.0. Eicosanoids were then extracted from aqueous phase with ethyl acetate. The ethyl acetate layer was neutralized with ammonium hydroxide. After evaporation of the ethyl acetate, eicosanoids were separated by thin layer chromatography (the organic phase resulting from the mixture of ethylacetate, isoctane, glacial acetic acid, water; vol/vol 110:50:20:100). Six-keto-PGF\(_{1\alpha}\) and TXB\(_2\) were measured by radioimmunoassay (RIA) using specific antibodies for them (Ono Pharmaceutical Co., Ltd.). The cross reaction rates with the 6-keto-PGF\(_{1\alpha}\) antiserum were 0.0% for TXB\(_2\), 9.1% for PGE\(_2\) and 5.0% for PGF\(_2\). Those with the TXB\(_2\) antiserum were less than 0.2% for all other eicosanoids. Intraassay coefficients of variation were less than 10% for both eicosanoids.

4) Check for platelet contamination in aortic preparation
To confirm whether or not platelets remain in the vessels after the aortic preparation, beta-thromboglobulin (TG) and platelet 4th factor (PF4), which are secreted from platelets, in aliquots after the incubation were measured by RIA.\(^8\)\(^9\)

5) Chemicals
\(^3\)H-6-keto-PGF\(_{1\alpha}\) and \(^3\)H-TXB\(_2\) were purchased from New England Nuclear. The antisera for 6-keto-PGF\(_{1\alpha}\) and TXB\(_2\), and standard 6-keto-PGF\(_{1\alpha}\) and TXB\(_2\) were provided by Ono Pharmaceutical Co., Ltd.. AA was purchased.
TABLE I  CHANGES IN EICOSANOIDS PRODUCTION BY AORTIC WALL OF SHR AND WKY WITH AGE

<table>
<thead>
<tr>
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<th>SHR</th>
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<th>WKY</th>
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<tbody>
<tr>
<td></td>
<td>Young (n=5)</td>
<td>Adult (n=9)</td>
<td>p</td>
<td>Young (n=5)</td>
</tr>
<tr>
<td>6-keto-PGF₁₀ (ng/100 mg)</td>
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<td></td>
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<tr>
<td>Basal (AA free)</td>
<td>294.1 ± 34.4</td>
<td>2258 ± 219</td>
<td>&lt; 0.001</td>
<td>234.6 ± 29.6</td>
</tr>
<tr>
<td>Maximal (AA 0.1 mM)</td>
<td>370.3 ± 31.1</td>
<td>5952 ± 377</td>
<td>&lt; 0.001</td>
<td>359.0 ± 36.0</td>
</tr>
<tr>
<td>Max/Maximal</td>
<td>1.38 ± 0.25</td>
<td>2.83 ± 0.48</td>
<td>&lt; 0.05</td>
<td>1.62 ± 0.24</td>
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<tr>
<td>TXB₂ (ng/100 mg)</td>
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<tr>
<td>Basal</td>
<td>30.3 ± 5.1</td>
<td>20.3 ± 3.1</td>
<td>ns</td>
<td>4.7 ± 1.1</td>
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</table>

In WKY, age-dependent remarkable increase in the synthesis of eicosanoids by aortic strips was observed. In contrast, arteries from SHR synthesized far more 6-keto-PGF₁₀ at the adult stage than at the young stage, but there was no significant difference in TXB₂ production between young and adult SHR.

from Sigma Chemical Co., Ltd., RIA kit of beta-TG was purchased from Radiochemical Centre Co., Ltd., and RIA kit of PF4 from Abbott Co., Ltd..

6) Statistical analysis
The values represent mean ± standard error. The difference was analyzed by paired or unpaired student’s t test.

RESULTS
1) Blood pressure
In 5-week-old SHR, age-matched WKY and 20 to 25-week-old WKY, systolic blood pressure ranged from 100 to 120 mmHg, but it ranged from 180 to 200 mmHg in 20 to 25-week-old SHR.

2) Platelet contamination in vessel wall
Beta-TG and PF4 in the buffer after the 45-min incubation were below identification limits (less than 7 ng/ml and 3 ng/ml, respectively).

3) Synthesis of eicosanoids in aortic wall
In 5-week-old SHR and WKY, aortic production of 6-keto-PGF₁₀ increased significantly with the addition of exogenous AA and reached a maximum when the concentration of AA was at 0.1 mM. No significant differences between SHR and WKY were found in the aortic 6-keto-PGF₁₀ production, either in the absence or presence of exogenous AA. In contrast, the amount of TXB₂ produced in aortic wall did not increase upon the addition of exogenous AA.

The aortic TXB₂ production was significantly greater in SHR as compared with WKY both in the absence of AA and in the presence of exogenous AA at the concentrations of 0.05, 0.1, and 0.3 mM. (Fig. 3, 4)

In 20 to 25-week-old SHR and WKY, aortic wall production of 6-keto-PGF₁₀ increased markedly when exogenous AA was added to the incubation buffer and reached a maximum in the presence of 0.1 mM AA. However, when the concentration of added exogenous AA was greater than 0.3 mM, 6-keto-PGF₁₀ synthesis was gradually inhibited in both SHR and WKY and fell below the basal level (AA free) when AA was greater than 5 mM. Aortic strips obtained from 20 to 25-week-old SHR synthesized more 6-keto-PGF₁₀ than those from WKY, both in the absence of AA and in the presence of AA at concentrations of 0.05, 0.1, 0.3, 0.6 and 1.0 mM. On the other hand, no differences were observed in TXB₂ formation between SHR and WKY at any AA concentration. The amount of TXB₂ produced in aortic wall increased slightly in the presence of AA at concentrations of 0.3 and 0.6 mM in WKY, but this was not observed in SHR. (Fig. 5, 6)

As shown in Table I, in WKY a remarkable increase in the aortic eicosanoid synthesis was observed as age increased; the production of 6-keto-PGF₁₀ increased by 6.2 fold in the absence of AA (basal), by 9.0 fold in the presence of AA (maximal) and TXB₂ production increased by 5.1 fold in a basal condition. Arteries from SHR synthesized far more 6-keto-PGF₁₀ in the adult stage than in the young stage.

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(7.7 fold in the basal condition and 16.1 fold in the maximal condition). However, there was no significant difference in TXB₂ production between young and adult SHR. The increase in basal and maximal vascular PGI₂ generation with age was significantly greater in SHR as compared with WKY. The maximal/basal 6-keto-PGF₁α production ratio increased with age in SHR, but did not in WKY.

**DISCUSSION**

Pace-Asciak et al.¹ have reported that the formation of PGI₂ in aortic rings and the conversion of AA to PGI₂ were enhanced with age in SHR. Botha et al.² provided that the generation of vascular PGI₂ was increased in adult SHR as compared with age-matched WKY. Uehara et al.³ clarified that this enhanced generation of vascular PGI₂ in SHR would be caused by an increased liberation of AA due to phospholipase C. Our results on vascular PGI₂ generation in SHR were in accord with the previous studies. However, there have been few studies concerning both the ability of vascular wall to produce PGI₂ and the effect of age on the production of vascular TXA₂ in SHR. It has been suggested that not only vascular PGI₂ generation but also TXA₂ metabolism in the aortic wall is very important to the regulation of vascular resistance since Wolfe et al.⁴ revealed that TXA₂ was formed from endogenous AA during incubation of rat’s aortic rings. Neri Serneri et al.⁵ reported that 47 ng/ml of beta-TG corresponded to the amount produced when a platelet count is below 5x10³/mm³, which could produce less than 50 pg/ml of TXB₂. In our study, the concentration of beta-TG after 45-minute incubation was less than 7 ng/ml. Thus, it was concluded that TXB₂ in the incubation mixture is produced by the vessel wall without significant contribution of the platelets.

A marked increase with age in basal production of eicosanoids was observed in WKY, and there was no difference between the percent increase in 6-keto-PGF₁α generation and that in TXB₂ generation. Thus, the synthesis of vascular eicosanoids in WKY was homogeneously enhanced with age, which can be explained by an increased liberation of AA. Since PGI₂ is known to inhibit cell growth⁶ it seems reasonable to presume that a low production of vascular PGI₂ in young rats will increase proliferative activity. In contrast to WKY, the SHR aortic strips synthesized only more 6-keto-PGF₁α at the adult stage. Moreover, the increase with age in basal and maximal vascular PGI₂ generation in SHR was significantly greater than WKY. The aortic TXB₂ production did not increase with age. These changes in the metabolism of vascular eicosanoids with age in SHR is thought to be related not only to aging but also to hypertension, because blood pressure elevates with age in SHR. Metabolic change in SHR aortic eicosanoids was not explained only by an enhanced liberation of AA from cellular membranes, because aortic 6-keto-PGF₁α production increased with age but TXB₂ generation did not. Accordingly, it is reasonable to presume that shift of TXA₂ to PGI₂ synthesis coexists with an increased liberation of AA in adult SHR, but its mechanism remains to be clarified.

Although the maximal/basal TXB₂ production ratio did not change significantly with age, the percent increase in 6-keto-PGF₁α production on the addition of exogenous AA was greater for adult SHR (183%) than for young SHR (38%). This difference in response to AA between young and adult SHR aortas suggests that the conversion of AA to PGI₂ may occur more easily in the adult aorta.

Aortic strips obtained from 20 to 25-week-old SHR synthesized more 6-keto-PGF₁α than those from WKY, both in the absence and presence of AA. These findings support the suggestion that elevation of PGI₂ levels may in part be an homeostatic response to hypertension, because depressor PGI₂ production increased and BP elevated. Although the mechanism of this enhanced production of vascular 6-keto-PGF₁α is still unclear, there seem to be at least two possibilities. Hypertension may directly influence vascular PGI₂ synthesis. Mechanical stimuli due to hypertension may enhance PGI₂ synthetase activity because PGI₂ synthetase is located in the cellular membranes.¹¹ The other is involvement of humoral or cell-structural alterations. In contrast to our results, Soma et al.¹² provided evidence for a decreased production of PGI₂ in SHR perfused mesenteric vascular beds. Thus, our results might not necessarily reflect PGI₂ production in the resistant vessels, and the exact mechanism or implications of the altered vascular PGI₂ production remains to be clarified.

In this study we clearly demonstrated that synthesis of TXB₂ in young SHR aortic strips was much higher than that in age-matched

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WKY. It has been reported that glomerular TXA₂ synthesis was increased in young SHR and that this enhanced production of TXA₂ may reduce renal function and increase arterial pressure. Our results seem to support that TXA₂ is important to the regulation of blood pressure in the vascular system. Indeed, there have been several studies which support these speculations. Shibouta et al. showed that the rise in arterial pressure of 4-week-old SHR was delayed for 1 week by chronic (3 weeks) therapy with the TXA₂ synthetase inhibitor CV4151, a pyridyl derivative. Uderman et al. reported an attenuated rise in arterial pressure in young SHR during therapy with an imidazole-derived TXA₂ synthetase inhibitor. Parkerson et al. demonstrated that arterial pressure in SHR was reduced to almost normal values during chronic treatment with the TXA₂ synthetase inhibitor OKY-046. In contrast to these findings, Grone et al. reported that acute or chronic administration of the TXA₂ synthetase inhibitor UK38845 had no significant effect on mean glomerular filtration rate or renal plasma flow in young SHR; arterial pressure was not influenced by inhibition of TXA₂ synthesis. These conflicting results may be related to differences in the duration of treatment and age at which treatment was initiated, but further work is needed.

Also, our data demonstrated clearly that the high concentration of AA inhibited the synthesis of vascular eicosanoids. This phenomenon is perhaps explained by damage of vascular cells, because in other studies scanning electron microscopical examination of the arterial wall revealed a high concentration of AA-damaged endothelial cells.

In summary, our findings show that the enhanced production of vascular PGI₂ was induced by both maturity and hypertension in SHR, and this increased synthesis could not be explained by the enhanced liberation of AA alone. Moreover, there is a possibility that the increased generation of TXA₂ in young SHR relates to the development of hypertension.

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