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

TOMOHIRO OSANAI, M.D., HARUO MATSUMURA, M.D., TAKASHI KIKUCHI, M.D.
OSAMU MINAMI, M.D., YOSHIKI YOKONO, M.D., RYUJI AKIBA, M.D.
HIROYUKI EIDOU, M.D., ATSUSHI KONTA, M.D., TAKEMICHI KANAZAWA, M.D.
KOGO ONODERA, M.D., HIROBUMI METOKI, M.D.*
AND YASABURO OIKE, M.D.*

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 0.1mM) 6-keto-PGF_{1α} 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-PGF_{1α} 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-PGF_{1α} was greater in SHR than in WKY. Moreover, the maximal/basal 6-keto-PGF_{1α} 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 PGF_{1α} but also a much greater reservoir of 6-keto-PGF_{1α} 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-Asciak et al¹ have reported an enhanced aortic synthesis of PGI₂ and increased conversion of AA to PGI₂ with age in SHR, and Botha et al² and Uehara et al³ have shown that generation of vascular PGI₂ increases

Key words:

SHR
WKY
Aorta
Prostacyclin
Thromboxane A₂

(Received April 3, 1989; accepted November 11, 1989)

Second Department of Internal Medicine, Hirosaki University School of Medicine, Hirosaki; *Reimeikyo Rehabilitation Hospital, Aomori, Japan

Mailing address: Tomohiro Osanai, M.D., Second Department of Internal Medicine, Hirosaki University School of Medicine, 5 Zaifucho, Hirosaki 036, Japan

Fig.1. Production of 6-keto-PGF_{1α} at each incubation time in rat's aorta.

The rat's aorta was incubated in tris HCl buffer (pH 7.5, 50 mM). The amount of 6-keto-PGF_{1α} produced by aortic wall increased with incubation time and reached its peak value at 45 min after the initiation of incubation.

Fig.2. Production of TXB₂ at each incubation time in rat's aorta.

The amount of TXB₂ produced by aortic wall increased with incubation time and reached its peak value at 30 min after the initiation of incubation.

in mature SHR as compared with age-matched Wistar Kyoto rats (WKY). Moreover, Lukacsko⁴ has provided the evidence that an aortic synthesis of PGI₂ gradually increased with age in both SHR and WKY, and that aortic strips from 20-week-old SHR synthesized more PGI₂ than vessels from age-matched WKY. However, it is still unclear whether or not there is a close relation between PGI₂ synthesis and elevation of blood pressure.

Recently, it has been reported that thromboxane A₂ (TXA₂), a potent platelet aggregating agent and vasoconstrictor, is also produced in the vascular tissue of rats⁵ and humans⁶. This evidence indicates that the vascular TXA₂ production in SHR may increase arterial pressure. However, there have been few studies on the ability of aortic vessels to produce PGI₂ and TXA₂ 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

Fig. 3. 6-keto-PGF_{1α} 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-PGF_{1α} formation in the aorta between 5-week-old SHR and WKY was observed. The amount of 6-keto-PGF_{1α} produced by aortic wall increased significantly on adding AA.

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

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.

(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-PGF_{1α}, a stable metabolite of PGI₂, and TXB₂, a stable metabolite of TXA₂.

2) Measurement of blood pressure in rats

Systolic blood pressures in all conscious rats were measured the day before sacrifice by the tailcuff method using a programmed electrophysiomonometer 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 method⁷ Crude lipid extracts were obtained from samples (0.3 ml) by the procedure of Folch. Nonlipid contaminations were

Fig. 5. 6-keto-PGF_{1α} production in the aorta of 20 to 25-week-old SHR and WKY with and without the addition of AA.

The rat's aorta was incubated in tris buffer for 45 min. The amount of 6-keto-PGF_{1α} produced by aortic wall was significantly greater in SHR as compared with WKY at AA concentration range from 0 to 1.0 mM. The synthesis of vascular 6-keto-PGF_{1α} reached the maximum on the addition of 0.1 mM AA into the buffer, but it was suppressed by adding more than 0.1 mM AA into the buffer.

Fig. 6. TXB₂ production in the aorta of 20 to 25-week-old SHR and WKY with and without the addition of AA.

Under the same experimental condition of 6-keto-PGF_{1α}, no difference was observed in TXB₂ formation between SHR and WKY. The amount of TXB₂ produced by aortic wall increased significantly in the presence of AA (0.3 and 0.6 mM) in WKY.

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, isooctane, glacial acetic acid, water; vol/vol 110:50:20:100). Six-keto-PGF_{1α} and TXB₂ were measured by radioimmunoassay (RIA) using specific antibodies for them (Ono Pharmaceutical Co., Ltd.). The cross reaction rates with the 6-keto-PGF_{1α} antiserum were 0.0% for TXB₂, 9.1% for PGE₂ and 5.0% for PGF₂. Those with the TXB₂ 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

³H-6-keto-PGF_{1α} and ³H-TXB₂ were purchased from New England Nuclear. The antisera for 6-keto-PGF_{1α} and TXB₂, and standard 6-keto-PGF_{1α} and TXB₂ 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

	SHR			WKY		
	Young (n=5)	Adult (n=9)	p	Young (n=5)	Adult (n=7)	p
6-keto-PGF _{1α} (ng/100 mg)						
Basal (AA free)	294.1 ± 34.4	2258 ± 219	< 0.001	234.6 ± 29.6	1452 ± 135	< 0.001
Maximal (AA 0.1 mM)	370.3 ± 31.1	5952 ± 377	< 0.001	359.0 ± 36.0	3240 ± 308	< 0.001
Max/Basal	1.38 ± 0.25	2.83 ± 0.48	< 0.05	1.62 ± 0.24	2.34 ± 0.27	ns
TXB ₂ (ng/100 mg)						
Basal	30.3 ± 5.1	20.3 ± 3.1	ns	4.7 ± 1.1	24.0 ± 5.0	< 0.01

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-PGF_{1α} 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 Abott 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-PGF_{1α} 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-PGF_{1α} 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-PGF_{1α} 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-PGF_{1α} 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-PGF_{1α} 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-PGF_{1α} 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-PGF_{1α} in the adult stage than in the young stage

(7.7 fold in the basal condition and 16.1 fold in the maximal condition). However, there was no significant difference in TXB_2 production between young and adult SHR. The increase in basal and maximal vascular PGI_2 generation with age was significantly greater in SHR as compared with WKY. The maximal/basal 6-keto- $\text{PGF}_{1\alpha}$ production ratio increased with age in SHR, but did not in WKY.

DISCUSSION

Pace-Asciak et al¹ have reported that the formation of PGI_2 in aortic rings and the conversion of AA to PGI_2 were enhanced with age in SHR. Botha et al² provided that the generation of vascular PGI_2 was increased in adult SHR as compared with age-matched WKY. Uehara et al clarified³ that this enhanced generation of vascular PGI_2 in SHR would be caused by an increased liberation of AA due to phospholipase C. Our results on vascular PGI_2 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 PGI_2 and the effect of age on the production of vascular TXA_2 in SHR. It has been suggested that not only vascular PGI_2 generation but also TXA_2 metabolism in the aortic wall is very important to the regulation of vascular resistance since Wolfe et al⁵ revealed that TXA_2 was formed from endogenous AA during incubation of rat's aortic rings. Neri Serneri et al⁶ reported that 47 ng/ml of beta-TG corresponded to the amount produced when a platelet count is below $5 \times 10^3/\text{mm}^3$, which could produce less than 50 pg/ml of TXB_2 . In our study, the concentration of beta-TG after 45-minute incubation was less than 7 ng/ml. Thus, it was concluded that TXB_2 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- $\text{PGF}_{1\alpha}$ generation and that in TXB_2 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 PGI_2 is known to inhibit cell growth,¹⁰ it seems reasonable to presume that a low production of vascular PGI_2 in young rats will increase proliferative activity. In contrast to WKY, the SHR aortic

strips synthesized only more 6-keto- $\text{PGF}_{1\alpha}$ at the adult stage. Moreover, the increase with age in basal and maximal vascular PGI_2 generation in SHR was significantly greater than WKY. The aortic TXB_2 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- $\text{PGF}_{1\alpha}$ production increased with age but TXB_2 generation did not. Accordingly, it is reasonable to presume that shift of TXA_2 to PGI_2 synthesis coexists with an increased liberation of AA in adult SHR, but its mechanism remains to be clarified.

Although the maximal/basal TXB_2 production ratio did not change significantly with age, the percent increase in 6-keto- $\text{PGF}_{1\alpha}$ 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 PGI_2 may occur more easily in the adult aorta.

Aortic strips obtained from 20 to 25-week-old SHR synthesized more 6-keto- $\text{PGF}_{1\alpha}$ than those from WKY, both in the absence and presence of AA. These findings support the suggestion that elevation of PGI_2 levels may in part be an homeostatic response to hypertension, because depressor PGI_2 production increased and BP elevated. Although the mechanism of this enhanced production of vascular 6-keto- $\text{PGF}_{1\alpha}$ is still unclear, there seem to be at least two possibilities. Hypertension may directly influence vascular PGI_2 synthesis. Mechanical stimuli due to hypertension may enhance PGI_2 synthetase activity because PGI_2 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 al¹² provided evidence for a decreased production of PGI_2 in SHR perfused mesenteric vascular beds. Thus, our results might not necessarily reflect PGI_2 production in the resistant vessels, and the exact mechanism or implications of the altered vascular PGI_2 production remains to be clarified.

In this study we clearly demonstrated that synthesis of TXB_2 in young SHR aortic strips was much higher than that in age-matched

WKY. It has been reported that glomerular TXA₂ synthesis was increased in young SHR¹³ 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 al¹⁴ 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 al¹⁵ reported an attenuated rise in arterial pressure in young SHR during therapy with an imidazole-derived TXA₂ synthetase inhibitor. Purkerson et al¹⁶ 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 al¹⁷ 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.¹⁸

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.

Acknowledgment

This work was supported in part by gifts from the Ono Pharmaceutical Co., Ltd..

REFERENCES

1. PACE-ASCIAC CR, CARRARA MC: Age-dependent increase in the formation of prostaglandin I₂ by intact and homogenized aortae from the developing spontaneously hypertensive rat. *Biochim Biophys Acta* 574: 177, 1979
2. BOTHA JH, LEARY WP, ASMAL AC: Enhanced release of a 'prostaglandin-like' substance from aortic strips of spontaneously hypertensive rats. *Prostaglandins* 19: 285, 1980
3. UEHARA Y, ISHIMITSU T, ISHII M, SUGIMOTO T: Prostaglandin synthase and phospholipases in the vascular wall of experimental hypertensive rats. *Prostaglandins* 34: 423, 1987
4. LUKACSKO P: Effect of arachidonic acid on the basal release of prostaglandin E₂ and I₂ by rat arteries during the development of hypertension. *Clin Exp Hypertens A5*: 1471, 1983
5. WOLFE LS, ROSTWOROWSKI K, MANKU M: Measurement of prostaglandin synthesis and release from rat aortic tissue and from the perfused mesenteric artery by gas chromatography-mass spectrometric methods. In *Prostaglandins* ed by VANE JR and BERGSTROM S, Raven Press, New York, 1979, p 113
6. NERI SERNERI GG, ABBATE R, GENSINI GF, PANETTA A, CASOLO GC, CARINI M: TXA₂ production by human arteries and veins. *Prostaglandins* 25: 753, 1983
7. INAGAWA T, OHKI S, SAWADA M, HIRATA F: Studies on extraction, separation and estimation of prostaglandins by radioimmunoassay. *Yakugaku Zasshi* 92: 1187, 1972 (in Japanese)
8. LUDLAM CA, MOORE S, BOLTON AE, PEPPER DS, CASH JD: The release of a human platelet specific protein measured by a radioimmunoassay. *Thromb Res* 6: 543, 1975
9. HANDIN RI, MCDONOUGH M, LESCH M: Elevation of platelet factor four in acute myocardial infarction: measurement by radioimmunoassay. *J Lab Clin Med* 91: 340, 1978
10. ISHIMITSU T, UEHARA M, ISHII M, SUGIMOTO T: Role of endogenous vasodilator prostaglandins in the proliferation of vascular smooth muscle cells of spontaneously hypertensive rats. *Clin Exp Hypertens A10*: 507, 1988
11. SMITH WL: Prostaglandin biosynthesis and its compartmentation in vascular smooth muscle and endothelial cells. *Ann Rev Physiol* 48: 251, 1986
12. SOMA M, MANKU MS, JENKINS DK, HORROBIN DF: Prostaglandins and thromboxane outflow from the perfused mesenteric vascular bed in spontaneously hypertensive rats. *Prostaglandins* 29: 323, 1985
13. KONIECKOWSKI M, DUNN MJ, STORK EJ, HASSID A: Glomerular synthesis of prostaglandins and thromboxane in spontaneously hypertensive rats. *Hypertension* 5: 446, 1983
14. SHIBOUTA Y, TERASHITA Z, INADA Y, NISHIKAWA K: Delay of the initiation of hypertension in spontaneously hypertensive rats by CV-4151, a specific thromboxane A₂ synthetase inhibitor. *Eur J Pharmacol* 109: 135, 1985
15. UDERMAN HD, WORKMAN RJ, JACKSON EK: Attenuation of the development of hypertension in spontaneously hypertensive rats by the thromboxane synthetase inhibitor, 4'-(imidazol-1-yl)acetophenone. *Prostaglandins* 24: 237, 1982

16. PURKERSON ML, MARTIN KJ, YATES J, KISSANE JM, KLAHR S: Thromboxane synthesis and blood pressure in spontaneously hypertensive rats. *Hypertension* 8: 1113, 1986
17. GRONE HJ, GRIPPO RS, ARENDSHORST WJ, DUNN MJ: Role of thromboxane in control of arterial pressure and renal function in young spontaneously hypertensive rats. *Am J Physiol* 250: F488, 1986
18. INGERMAN-WOJENSKI C, SILVER MJ, SMITH JB, NISSENBAUM M, SEDAR AW: Prostacyclin production in rabbit arteries in situ: inhibition by arachidonic acid-induced endothelial cell damage or low-dose aspirin. *Prostaglandins* 21: 655, 1981