ALTERATIONS OF THE CARDIOVASCULAR AND RENAL PROSTAGLANDINS AND THROMBOXANES SYSTEM IN PREHYPERTENSIVE SPONTANEOUSLY HYPERTENSIVE RATS

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To assess the participation of cardiovascular eicosanoids (prostaglandins and thromboxanes) system in the initiation of genetic hypertension, we examined eicosanoids metabolism in the heart, aortic wall and kidney in prehypertensive and hypertensive rat models for spontaneous hypertension (SHR). Vasoconstrictor thromboxane A_2 (TXA_2) generation in the aortic wall was significantly enhanced by 49% in the prehypertensive and by 18% in the hypertensive SHR when compared to the respective normotensive Wistar-Kyoto rats. Cardiac TXA_2 content was significantly increased as well by 14% in the prehypertensive and by 30% in the hypertensive SHR. Moreover, vascular vasodepressor eicosanoids generation was decreased by 10% for PGI_2 and by 29% for PGD_2 in the prehypertensive SHR although the alterations were eliminated in the hypertensive SHR. In contrast to the cardiovascular eicosanoids system, there was no difference in renocortical TXA_2 content in either young or adult SHR while vasodepressor prostaglandins contents were decreased by 29% for PGE_2 and by 33% for PGD_2 in SHR when they were in the prehypertensive stage. Thus, in the prehypertensive stage of SHR, the cardiovascular eicosanoids system exhibited enhanced vasoconstrictor TXA_2 and decreased vasodepressor prostaglandins, thereby producing a vasoconstrictor state. These data indicate that the alterations in the cardiovascular eicosanoids system partially contribute to the initiation of hypertension in SHR.

EICOSANOIDS (prostaglandins and thromboxanes) have been demonstrated to participate in blood pressure regulation through direct effects on vascular smooth muscle contractility or body fluid homeostasis.¹⁻⁴ Indeed, there is some evidence that Dahl salt sensitive

rats (Dahl S rats), a genetic model for salt-induced hypertension, have alterations in the vascular eicosanoids system, e.g., lowered vasodepressor prostaglandins and enhanced vasoconstrictor thromboxane A_2 (TXA_2).⁵⁻⁷ However, conflicting data have been reported concerning the basal cardiovascular eicosanoids system in genetic rat models for spontaneous hypertension (SHR).⁸⁻¹⁰ Pace-Asciak et al⁹ have demonstrated that prostacyclin (PGI_2) generation in the aortic wall is enhanced in SHR,

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whereas Soma et al¹¹ provide evidence that vasodepressor prostaglandins generation is lowered in SHR, using perfused mesenteric arterial beds.

In this context, we have found that high blood pressure is a potential activator of vascular prostacyclin synthase, thereby increasing vascular PGI₂ generation in various experimental models for hypertension.¹² This mechanism is likely to mask the basal arachidonate metabolism.

In order to assess the roles of cardiovascular eicosanoids system in the development of hypertension, it seems more appropriate to investigate arachidonate metabolism when SHR are in the prehypertensive stage, where the effects of blood pressure are minimized. Thus, in this study, we examined cardiovascular eicosanoids system in 5-week-old SHR which are absolutely in the prehypertensive stage and compared the alterations with those in 15-week-old, hypertensive SHR.

MATERIALS AND METHODS

Twenty 5-week-old male SHR and 20 age-matched male Wistar-Kyoto rats (WKY) were used in this study. In comparison, ten 15-week-old male SHR and 10 age-matched male WKY were also investigated. They were bred at the 2nd Department of Medicine, University of Tokyo, Tokyo, Japan. After weaning, they were fed a 0.6% NaCl diet. Water was given ad libitum. Systolic blood pressure was measured by the tail-cuff method. The descending thoracic aorta, heart and kidney were removed under intraperitoneal Inactin anesthesia (100 mg/kg).

Vascular Capacity to Generate Eicosanoids

To investigate the metabolism of arachidonate, the capacity of the vascular wall to generate eicosanoids was measured.⁵ Briefly, the descending thoracic aorta was placed in an ice-chilled Dulbecco's phosphate buffered saline solution (D-PBS, Gibco Laboratory, Grand Island, New York, U.S.A.). A 50 mm² aortic strip was carefully prepared so that mechanical stimuli were avoided. The strip was incubated in 2 ml of oxygenized D-PBS at pH 7.35. Vasodepressor prostaglandins, e.g. PGI₂, PGE₂ and PGD₂ and vasoconstrictor TXA₂, which were released in the incubation medium at 37°C for 30 min, were measured by the direct radioimmunoassay.^{5,12} The strip was dried at 80°C for 48 hours for the measurement of dry tissue weight of the aorta.

Cardiac Content of Eicosanoids

The hearts were quickly rinsed with ice-chilled D-PBS to remove any residual blood present in the ventricles. The washed hearts were frozen in liquid nitrogen, and stored under -70°C until the extraction procedure. The frozen tissue was warmed up to -5°C. Only ventricles were used for the following studies. The excised tissue was homogenized with a Potter's homogenizer in a chloroform-methanol solvent (2 : 1, v/v) cooled with dry ice. The homogenate was passed through Whatman 541 filter paper. The effluent was evaporated to dryness, and dissolved with 20% ethanol (v/v) in 0.2M citrate-buffered solution at pH 3.5. The residue on the paper was dried at 80°C for 48 hours, and weighed.

Eicosanoids were purified according to the method of Powell.¹³ Briefly, each sample was applied to a Sep-Pak (C18) cartridge (octadecylsilyl-silica cartridge, Waters Associates, Milford, Massachusetts, U.S.A.), which was prewashed with 20 ml of absolute methanol and 20 ml of distilled water, and then equilibrated with 40 ml of 20% ethanol (v/v) in 0.2M citrate-buffered solution at pH 3.5. The loaded cartridge was washed successively with 20 ml of distilled water, 20 ml of 20% chloroform in n-hexane (v/v) and 20 ml of n-hexane. Finally, eicosanoids were eluted with 3 ml of methyl formate. The purified eicosanoids were evaporated to dryness, and dissolved in 1 ml of 50 mM phosphate buffered solution for radioimmunoassay. The recovery rates averaged 64.4 ± 2.2%. The extracted and purified eicosanoids were measured by radioimmunoassay.^{5,12} The measurement was corrected by the respective recovery rates and then related to the dry fat-free tissue weight.

Renal Content of Eicosanoids

The isolated kidneys were immediately perfused with ice-chilled 0.38% citrated, calcium and magnesium-free D-PBS. The residual blood was completely washed out. The kidney was quickly plunged into liquid nitrogen. The frozen tissue was warmed up to -5°C, and then sectioned at a width of 0.5 mm. The outer cortex was punched out and processed in the same way as the cardiac ventricles. The average recovery rate was 63.3 ± 2.7%. Eicosanoids were measured by radioimmunoassay.^{5,12} The measurement was corrected by the respective recovery rates and related to the dry fat-free tissue weight.

TABLE I PRODUCTION OF VARIOUS EICOSANOIDS IN THE AORTIC WALL OF SHR AND WKY

Strains	PGI ₂	PGE ₂	PGD ₂	TXA ₂
<i>5-Week-Old:</i>				
SHR (20)	2.98 ± 0.30	0.225 ± 0.018	0.252 ± 0.033*	0.365 ± 0.026**
WKY (20)	3.57 ± 0.45	0.240 ± 0.037	0.356 ± 0.034	0.245 ± 0.016
<i>15-Week-Old:</i>				
SHR (10)	32.3 ± 3.69**	0.260 ± 0.019*	0.209 ± 0.024	0.350 ± 0.016*
WKY (10)	17.6 ± 1.00	0.197 ± 0.011	0.197 ± 0.015	0.297 ± 0.014

Values are expressed as $\mu\text{g/g}$ dry weight of aorta/30 min. A number in the parenthesis represents the number of studies. * $p < 0.025$, ** $p < 0.005$ vs the respective values of age-matched WKY.

Fig.1. Content of Various Eicosanoids in the Cardiac Ventricles of SHR and WKY. The left graph shows 5-week-old SHR and WKY, and the right graph 15-week-old SHR and WKY. The open and closed bars represent WKY and SHR, respectively. NS represents statistically not significant.

Radioimmunoassay

Radioimmunoassay was performed according to the previous method⁵. Briefly, the assay mixture consisted of 0.1 ml of (³H) eicosanoid (10^4 dpm), 0.1 ml of a diluted sample or standard solution, and 0.1 ml of a diluted antibody solution. The assay mixture was incubated at 4°C for 24 hours. To separate the bound from the free (³H) eicosanoid, 0.1 ml of a dextran-coated charcoal solution (2.5% charcoal and 0.25% dextran) was added and vortexed. The mixture was immediately centrifuged at 1000g at 4°C for 5 min. The radioactivity of the (³H) eicosanoid bound to the antibody was measured by an automatic liquid scintillation counter. The antisera were raised in rabbits in our laboratory, using eicosanoid-coupled thyroglobulin and

Freund's complete adjuvant as the immunogen. The immunization was repeated every 2 weeks for 4 months. Anti-6-keto-PGF_{1α} serum crossreacted 1% with PGE₂, 2% with PGF_{2α}, 0.08% with PGD₂, 0.01% with TXB₂ and less than 0.001% with arachidonate. Anti-TXB₂ serum crossreacted 0.005% with 6-keto-PGF_{1α}, 0.007% with PGE₂, 0.018% with PGF_{2α}, 0.06% with PGD₂ and less than 0.0001% with arachidonate. Anti-PGE₂ serum crossreacted 0.4% with 6-keto-PGF_{1α}, 0.5% with PGF_{2α}, 0.1% with PGD₂, 0.02% with TXB₂, and less than 0.001% with arachidonate. Anti-PGD₂ serum crossreacted 0.01% with 6-keto-PGF_{1α}, 0.01% with PGE₂, 0.2% with PGF_{2α}, 0.01% with TXB₂ and less than 0.001% with arachidonate.

Fig. 2. Content of Various Eicosanoids in the Renocortices of SHR and WKY. The left graph shows 5-week-old SHR and WKY, and the right graph 15-week-old SHR and WKY. The open and closed bars represent WKY and SHR, respectively. NS represents statistically not significant.

Reagents

The reagents were all of analytical grade. The authentic eicosanoids were supplied gratis by Ono Pharmaceutical Co., Ltd., Osaka, Japan. (³H) eicosanoids were purchased from Amersham, Japan.

Statistical Analysis

The values are expressed as mean $\pm$ SE. The difference was analyzed by the Student's *t* test.

RESULTS

Although 5-week-old SHR remained normotensive, the average systolic blood pressure was 10 mmHg higher than age-matched control WKY (119 ± 2 vs 109 ± 3 mmHg, $p < 0.01$). The 15-week-old SHR developed established hypertension, with the difference in blood pressure between the two strains becoming much greater (189 ± 4 vs 134 ± 3 mmHg, $p < 0.01$).

The capacity of the aortic wall to generate eicosanoids is depicted in Table I. The major metabolite of arachidonate was PGI₂, which was in accord with our previous study⁵. The aortic wall produced TXA₂, PGE₂ and PGD₂ as well, although the amounts were much lower than PGI₂. Vasoconstrictor TXA₂ generation was enhanced significantly by 49% in prehypertensive and by 18% in hypertensive SHR. Vasodepressor PGD₂ production was significantly reduced by 29% and the other vasodepressor prostaglandins

tended to be reduced as well in 5-week-old SHR when compared to the controls. In contrast, vasodepressor PGI₂ and PGE₂ production was significantly increased in 15-week-old, hypertensive SHR by 84% and by 32%, respectively.

Figure 1 shows the cardiac content of eicosanoids in 5-week-old and 15-week-old SHR and WKY. Cardiac TXA₂ content was significantly increased by 14% in prehypertensive (21.3 ± 0.9 vs 18.7 ± 1.1) and by 30% in hypertensive SHR (12.1 ± 0.9 vs 9.3 ± 0.7 ng/g fat free tissue weight) when compared to the respective controls. The content of vasodepressor prostaglandins was not changed in either prehypertensive or hypertensive SHR.

Figure 2 presents the content of eicosanoids in the renal cortex of prehypertensive and hypertensive SHR and WKY. In either prehypertensive or hypertensive SHR, there were no changes in vasoconstrictor TXA₂ content. In contrast, vasodepressor PGE₂ and PGD₂ contents in the prehypertensive stage of SHR were significantly decreased by 29% (72 ± 9 vs 102 ± 10) and by 33% (33.7 ± 5.4 vs 50.2 ± 6.9 ng/g fat free tissue weight), respectively, when compared to the controls. The alterations were completely abolished in hypertensive SHR.

DISCUSSION

In this study, we measured the capacity of the aortic wall to generate eicosanoids in order to

assess the eicosanoids system in the vascular wall. Eicosanoids generated in this assay system have been shown to reflect largely the productive aspect of arachidonate metabolism.⁵ In order to investigate the cardiac and renal eicosanoids system, we measured the tissue content of eicosanoids, using the quick-frozen technique according to the method of Tobian et al.⁶ Since the cells are supposed to have little capacity to store synthesized eicosanoids, and because the eicosanoids are quickly released from the cells, the tissue content may largely reflect eicosanoids in the extracellular surroundings.¹⁴ In addition, since eicosanoids are degraded locally in the tissues, the tissue content of eicosanoids is probably influenced by their breakdown as well.

One of the most important findings in this study is that cardiovascular TXA₂ system of SHR is enhanced when they are still in the prehypertensive stage. The enhanced TXA₂ system was observed as well even after they developed established hypertension, which is in agreement with previous studies.^{10,15} TXA₂ is a potent vasoconstrictor and is possibly involved in smooth muscle cell proliferation.¹⁶ The alteration of the cardiovascular TXA₂ system in SHR conceivably contributes to the vasoconstrictor state and to the increase of vascular wall thickness, both of which are integral components of spontaneously hypertensive rats.¹⁷ The roles of cardiac TXA₂ in the development of hypertension are unclear. However, since TXA₂ is exerted to facilitate cell growth,¹⁶ the alteration of cardiac TXA₂ may be related to some extent to the premature cardiac hypertrophy of SHR.

There is much evidence that vasodepressor prostaglandins are increased in hypertensive, adult SHR. It has been interpreted as an adaptive phenomenon to compete with the blood pressure elevation.^{8,9} In addition, blood pressure elevation is found to be potential activator of PGI₂ synthase.¹² Thus, in order to investigate the basal eicosanoids system, it seems necessary to minimize the possible influence of high blood pressure. In this context, we clearly demonstrated, using very young SHR which remained normotensive, that lowered vasodepressor prostaglandins, e.g. PGI₂, PGE₂ and PGD₂, in the vascular wall preceded the development of hypertension. The impaired vasodepressor prostaglandins system is possibly masked by the development of hypertension in adult SHR. Seemingly, our hypothesis is supported by the previous study of Soma et al.¹¹ which describes

an impaired vasodepressor prostaglandins outflow in the perfused mesenteric arterial beds of SHR, or the study of Lukacsko et al.¹⁸ which demonstrates that the vasodepressor response of arachidonate is lowered in SHR. If the basal vasodepressor prostaglandins system declines somewhat in SHR, the alterations may be at least partly responsible for the vasoconstrictor state or increase in vascular wall thickness,^{16,19} both of which are observed even in prehypertensive SHR.

In the renocortical tissue, there was no difference in TXA₂ content between the two strains. However, vasodepressor PGE₂ was significantly decreased in the prehypertensive stage of SHR. Vasodepressor PGE₂ retards sodium reabsorption in the cortical collecting ducts.²⁰ The decreased PGE₂ content in the renocortex facilitates the reabsorption of sodium, thereby contributing to sodium retention. In addition, PGD₂ content was significantly lowered in the renocortex of young SHR as well. The reduced vasodepressor PGE₂ and PGD₂ possibly declines renal blood flow, thereby producing sodium retention again. Thus, the alterations of vasodepressor prostaglandins in prehypertensive SHR possibly participate in the initiation of genetic hypertension in SHR. There is some evidence that the renomedullary eicosanoids system is activated in hypertensive, adult SHR.²¹⁻²³ However, there are few data available concerning renomedullary vasodepressor prostaglandins in prehypertensive, young SHR.

Finally, the mechanisms for the altered cardiovascular TXA₂ system remain to be elucidated. The enhanced TXA₂ and impaired vasodepressor system strongly indicate that the altered cardiovascular TXA₂ system could not be explained only by the common enzymatic steps in arachidonate cascade, but also by TXA₂ synthase. Since the enhanced TXA₂ system is observed in cultured vascular smooth muscle cells in SHR¹⁶ as well, it seems highly possible that the alterations are genetically expressed in SHR. To address these questions more directly, sub-cellular analysis of vascular smooth muscle cells in SHR is required.

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