Prostacyclin and Thromboxane Biosynthesis in Mild Essential Hypertension

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The possibility that prostacyclin or thromboxane biosynthesis is abnormal in patients with established mild essential hypertension was investigated in 46 patients. These eicosanoids have opposing effects both on vascular smooth muscle and on platelets. An imbalance in their biosynthesis could therefore influence both vascular tone and predisposition to thrombosis. We studied the relation between blood pressure and the biosynthesis of prostacyclin and thromboxane A2 by measuring urinary excretion rates of stable breakdown products of prostacyclin (6-oxo-prostaglandin F1a, and 2,3-dinor-6-oxo-prostaglandin F1a) and of thromboxane A2 (thromboxane B2 and 2,3-dinor-thromboxane B2) using immunoaffinity chromatography and gas chromatography/electron capture mass spectrometry. Excretion rates of both of the prostacyclin-derived products ranged from less than 5 to more than 100 ng/g creatinine; each was significantly negatively correlated with blood pressure (r=0.36-0.45). A reduction of 2,3-dinor-6-oxo-prostaglandin F1a excretion of 100 ng/g creatinine was associated with an increase in arterial pressure of 14 mm Hg (systolic) and 8 mm Hg (diastolic) in patients who had been without antihypertensive medication for 2 weeks. The same reduction in 6-oxo-prostaglandin F1a excretion was associated with an increased pressure of 19 mm Hg (systolic) and 12 mm Hg (diastolic) (2p<0.05 for diastolic pressure and 2p<0.01 for systolic pressure in each case). There were similar correlations between the excretion rates of these products and blood pressure in the same patients while they were receiving antihypertensive therapy. In contrast, excretion rates of thromboxane B2 and 2,3-dinor-thromboxane B2 were not significantly correlated with blood pressure. We conclude that prostacyclin biosynthesis is selectively impaired in mild essential hypertension. This could contribute to the raised peripheral resistance and increased incidence of thrombosis that are characteristic of essential hypertension. (Hypertension 1990;15:469-474)
what oversimplified. We have measured these products in urine from patients with mild essential hypertension to determine the relation between blood pressure and PGF₂ and TXA₂ biosynthesis.

Methods

Forty-six patients (24 male and 22 female, 31–54 and 35–60 years of age, respectively) gave written, informed consent to participate in the study. The protocol was approved by the Ethics Committee of Hammersmith and Queen Charlotte's Hospitals. Each patient had been diagnosed as hypertensive on the basis of five or more readings of diastolic blood pressure 90 mm Hg or higher over a period of 6 weeks or longer. None had had diastolic pressures higher than 118 mm Hg, or systolic pressures higher than 200 mm Hg. Seven patients (five men, two women) regularly smoked 10 cigarettes or more per day, and the remainder were nonsmokers. At the time of recruitment, all but one were receiving antihypertensive therapy. Treatment was with diuretic alone (six patients), β-blocker alone (four patients), diuretic and β-blocker together (21 patients), or vasodilator with diuretic or β-blocker (14 patients). There was no evidence of secondary hypertension on the basis of history, physical examination, urinalysis, and urinary vanillyl mandelic acid excretion (<35 μg/ml/24 hr on one or more occasions). Serum creatinine concentrations were all within the normal range of our laboratory (70–125 μmol/l). No aspirin or other nonsteroidal anti-inflammatory drugs were taken for 2 weeks or more before the study; compliance was confirmed by measurement of the capacity of platelets to synthesize thromboxane by incubation of whole blood in plain glass tubes at 37° C and measurement of TXB₂ in the resulting serum as described by Patrono et al.¹⁴ This measurement was not available in four samples because of inadvertent heating of the water bath to 52° C. Subjects collected urine for a 24-hour period from 8:00 AM to 8:00 AM for prostaglandin, creatinine, and electrolyte (Na⁺ and Ca²⁺) determinations. Blood pressure was measured in triplicate using an automated indirect instrument (Dinamap, Critikon, Ascot, Berkshire, UK) between 10:00 and 11:00 AM on the day of the urine collection after 60 minutes of supine rest in a quiet room. A 10 ml venous blood sample was taken for determination of plasma creatinine and electrolytes. Antihypertensive medication was discontinued, and no other drugs were taken during the following 2 weeks. Urine and blood were collected, and blood pressure determinations were then made as before. Diet was unmodified throughout. Twenty-four-hour urine collections were made similarly in 30 normotensive subjects (blood pressure <140/80 mm Hg; 15 male and 15 female, 28–61 and 31–52 years old, respectively) for determination of prostaglandins.

Analysis

Prostaglandins and thromboxanes were assayed in urine using immunoaffinity chromatography and capillary column gas chromatography/electron capture mass spectrometry as described in detail elsewhere.¹⁵,¹⁶ Briefly, urine samples (10 ml) were diluted 1:1 by volume with buffer at pH 8.0 and [¹H₆]-6-oxo-PGF₁α, [¹H₂]-TXB₂, [¹H₆]2,3-dinor-6-oxo-PGF₁α, and [¹H₆]2,3-dinor-TXB₂ (5 ng each) were added. Samples were stored at −20°C. Prostaglandins and thromboxanes were extracted with cyanogen bromide–activated sepharose columns containing immobilized antibodies that had been raised against 6-oxo-PGF₁α and TXB₂ and that cross-reacted with 2,3-dinor-6-oxo-PGF₁α and 2,3-dinor-TXB₂. Urine samples were applied under vacuum to the columns, which were washed with water (10 ml). Eicosanoids were eluted by addition of acetone–water (95:5; 0.5 ml) and rotating the columns for 15 minutes. Samples were taken to dryness (N₂ stream) and were derivatized as 3,5-bistrifluoromethylbenzyl esters and trimethylsilyl ethers.¹⁷ They were stored in n-dodecane (15 μl) with desiccant. Aliquots (2.5 μl) were analyzed with a Finnigan 4500 GC/mass spectrometer (Finnigan, San Jose, California) in the electron capture mode using ammonia as reagent gas. Carboxylate anions at mass-to-charge ratio (m/z) 585 were monitored for 6-oxo-PGF₁α and TXB₂ and at m/z 589 for their deuterated internal standards. Ions at m/z 557 and 561 were monitored simultaneously for the 2,3-dinor metabolites and their deuterated internal standards, respectively. The detection limit for each eicosanoid was 5 pg/ml when 10 ml urine samples were assayed.

Urine pH was recorded. Plasma and urinary creatinine, Na⁺, and Ca²⁺ were measured using routine methods in the Chemical Pathology Laboratory at Hammersmith Hospital.

Data Analysis

The mean of triplicate blood pressure readings was used to compare with eicosanoid excretion rate. Correlations were sought using the method of least-squares linear regression and considered significant when 2p<0.05. Results are expressed as mean (SD of the mean).

Results

Eicosanoid and electrolyte excretion data are summarized in Table 1. After the 2-week washout period, there was a significant negative correlation between 2,3-dinor-6-oxo-PGF₁α excretion and both systolic (r=0.45; 2p<0.01) and diastolic (r=0.36; 2p<0.05) blood pressure (Figure 1, bottom left). The slopes of the regression lines were such that a decrease of 2,3-dinor-PGF₁α excretion of 100 ng/g creatinine was associated with a rise in diastolic pressure of 13.9 mm Hg and a rise in systolic pressure of 7.9 mm Hg. Similarly, there was a significant negative correlation between 6-oxo-PGF₁α excretion and both systolic (r=0.42; 2p<0.01) and diastolic (r=0.36; 2p<0.05) pressures (Figure 1, bottom right). The slopes of these regression lines were 19.0 and 11.7 mm Hg/100 ng/g creatinine decline in 6-oxo-PGF₁α excretion for
systolic and diastolic pressures, respectively. While patients were receiving antihypertensive treatment, systolic and diastolic blood pressures were also significantly negatively correlated with excretion of both 2,3-dinor-6-oxo-PGF$_{1\alpha}$ ($r=0.42$, 2$p<0.01$ systolic; $r=0.37$, 2$p<0.05$ diastolic) and 6-oxo-PGF$_{1\alpha}$ ($r=0.34$, 2$p<0.05$ systolic; $r=0.35$, 2$p<0.05$ diastolic) (Figure 1, top, left and right panels, respectively). The ordinate intercepts were lower during treatment: 134.7/84.2 mm Hg (systolic/diastolic) for the 2,3-dinor-6-oxo-PGF$_{1\alpha}$ regression lines and 134.0/84.1 mm Hg for the 6-oxo-PGF$_{1\alpha}$ lines, as compared with corresponding untreated values of 158.8/97.8 and 160.0/98.8 mm Hg. The slopes of the regression lines from the data during treatment were 5.5 mm Hg (systolic) and 3.2 mm Hg (diastolic)/100 ng 2,3-dinor-6-oxo-PGF$_{1\alpha}$/g creatinine excreted and 6.5 mm Hg (systolic) and 4.4 mm Hg (diastolic)/100 ng 6-oxo-PGF$_{1\alpha}$/g creatinine. In contrast, neither 2,3-dinor-TXB$_2$ nor TXB$_2$ excretion was significantly related to systolic or diastolic arterial pressures either during antihypertensive treatment or after washout (Figure 2) (2$p>0.1$ in all cases). Mean serum TXB$_2$ was 226.7±83.5 ng/ml after the 2-week washout period and 212.6±90.9 ng/ml while receiving antihypertensive treatment. There was no significant correlation between eicosanoid excretion and age, serum TXB$_2$, urine pH, sodium or calcium excretion either with or without antihypertensive therapy.

### Discussion

McGiff and Vane$^{18}$ in 1975 suggested that prostaglandins participate in the regulation of blood pressure by exerting local actions both within the kidney and within arterial walls. They suggested that prostaglandin synthesis within arterial walls could oppose hormonal and nervous-induced vasoconstriction and thereby affect the tone of resistance vessels. They further pointed out that some cyclooxygenase products can increase blood pressure. Disordered prostaglandin synthesis could therefore contribute to the
The discovery and biological properties of PGI₂ and TXA₂ raised the possibility that reduced PGI₂ synthesis or increased TXA₂ synthesis could contribute to the pathophysiology of essential hypertension. This is attractive because, unlike PGE₂ and PGF₂α, these eicosanoids influence platelet function as well as vascular tone, and thrombotic events are the major cause of excess mortality in patients with mild essential hypertension. Several animal models of hypertension have been studied and have been reviewed recently. The results have varied widely from model to model. A study of one model of possible relevance to human essential hypertension, the salt-sensitive Dahl rat, demonstrated reduced excretion of 2,3-dinor-6-oxo-PGF₁α compared with the normotensive control strain. Interestingly, impaired PGI₂ synthesis preceded the development of hypertension, so in this model, impaired PGI₂ synthesis cannot be secondary to raised blood pressure.

There have been several studies of TXA₂- or PGI₂-derived products in human essential hypertension. Simultaneous measurements of excretion rates of 6-oxo-PGF₁α, TXB₂, and their 2,3-dinor metabolites in such patients have not, however, been reported previously. In the present study, we found no absolute differences between the urinary excretion of these eicosanoids in mild hypertensive and control subjects. The similar TXB₂ excretion rates in hypertensive and normotensive groups differ from findings reported by Hornych et al who found increased TXB₂ excretion in patients with mild essential hypertension and in agreement with the findings of Campbell et al who observed no consistent difference between urinary TXB₂ excretion in normal subjects and hypertensive patients. In view of the unimodal distribution of blood pressure in the population, it is somewhat arbitrary to divide individuals into distinct hypertensive and normotensive groups. Instead, the present findings suggest that there is a continuous relation between PGI₂ biosynthesis and blood pressure in patients with mild hypertension by demonstrating significant negative correlations between arterial pressure (both systolic and diastolic) and the rates of excretion of both 6-oxo-PGF₁α and 2,3-dinor-6-oxo-PGF₁α. The correlation coefficients suggest that differences in PGI₂ synthesis account for only a small part of the observed variation in blood pressure. We would not have expected to have observed effects of much greater magnitude than this in view of the many other factors independent of prostaglandin synthesis that influence blood pressure acutely, notably stress and anxiety. We attempted to reduce such variability by using carefully standardized conditions for blood pressure measurement (after 1 hour of supine rest and at the same time of day) in all patients. It is possible that, in future studies, it will be possible to improve the measurement of blood pressure still further, perhaps by the use of ambulatory blood pressure recording.

It is not possible from the present data to determine whether the negative relation between excretion rates of PGI₂-derived products and blood pressure extends into the normotensive range because the range of blood pressures in the normotensive subjects was too compressed to justify correlation analysis. We considered the possibility that the findings reflect impaired excretion of eicosanoid metabolites in progressively severe hypertensive subjects, but we found no relation between prostaglandin excretion and creatinine clearance, sodium excretion, or urine pH. Furthermore, the relation between blood pressure and eicosanoid excretion was specific for PGI₂-derived and not for TXA₂-derived products. The lack of correlation of excretion rates of thromboxane-derived products with blood pressure also argues against the possibility that inadvertent use of cyclooxygenase inhibitors by some of the patients could have explained the significant negative correlations of each of the PGI₂-derived products with blood pressure, as cyclooxygenase inhibition would suppress thromboxane synthesis to as great (or greater) an extent as PGI₂ synthesis. Furthermore, serum TXB₂ values were very similar to those reported by Patrono et al in healthy drug-free subjects, whereas aspirin and other cyclooxygenase inhibitors reduce serum TXB₂ very substantially. In four subjects, serum TXB₂ was not measured because of inadvertent overheating of the samples. However, urinary 6-oxo-PGF₁α and 2,3-dinor-6-oxo-PGF₁α in these subjects was in the upper two thirds of the range, and omission of these data did not affect the significance of the correlations. We therefore conclude that the higher the blood pressure in hypertensive subjects, the lower the rate of biosynthesis of PGI₂ both within the kidney and also elsewhere in the body. We cannot determine whether the decrement in PGI₂ biosynthesis is a primary event (as in the Dahl rat) or is secondary to the development of hypertension: to do so will require longitudinal or family studies, but in either case, impairment of PGI₂ biosynthesis in essential hypertension could have important pathophysiological consequences.

FitzGerald et al demonstrated that there is an increase in 2,3-dinor-6-oxo-PGF₁α excretion during pregnancy and showed that this is blunted in women who subsequently developed pregnancy-induced hypertension. They concluded that decreased PGI₂
formation might play a part in pregnancy-induced hypertension; the present study provides evidence that this may also be true of essential hypertension. Knapp and FitzGerald27 measured 2,3-dinor-6-oxo-PGF1α excretion in patients with essential hypertension in a study primarily directed toward elucidating the antihypertensive effect of fish oil. Correlation of the excretion rate of this metabolite with blood pressure, as reported in the present study, was not presented, but the rate of 2,3-dinor-6-oxo-PGF1α excretion was similar to values in normotensive subjects, which is consistent with our finding of similar mean excretion rates in groups of mildly hypertensive and normotensive individuals. Beckmann et al.40 however, found that 2,3-dinor-6-oxo-PGF1α excretion was significantly less in a group of 10 patients with mild essential hypertension than in normal volunteers and also reported that in a subgroup characterized as “responders” to the antihypertensive effect of propranolol, but not in the entire group, there was a negative correlation between 2,3-dinor-6-oxo-PGF1α excretion and blood pressure. We did not address the question of responsiveness to propranolol or other drugs in our patients, but the group as a whole qualitatively similarly as regards the relation between 2,3-dinor-6-oxo-PGF1α excretion and blood pressure to the propranolol responders in the study by Beckmann et al.40 These findings suggest that if an individual becomes hypertensive the rate of biosynthesis of PG12 determines, in part, the severity of the hypertensive state. Several vasoconstrictor hormones, including angiotensin II, vasopressin, and epinephrine, stimulate PG12 synthesis by cultured vascular cells and by intact vascular tissue in vitro.41,42 If vasoconstrictors stimulate PG12 synthesis in vivo, this could constitute a homeostatic mechanism, the sensitivity or gain of which might vary between individuals. Those least able to increase PG12 synthesis in response to pressor stimuli would be expected to manifest the highest blood pressures, accounting for the negative correlations that we observed. Whatever the mechanism underlying the diminishing rate of PG12 production with increasing arterial pressure, and irrespective of whether this is a primary abnormality or is secondary to the raised arterial pressure, a relative deficiency of PG12 compared with TXA2 could influence not only vascular resistance, but also platelet aggregation and hence the predisposition to arterial thrombosis in mild essential hypertension. Because dietary measures or drugs, notably thromboxane synthase inhibitors, can reduce the biosynthesis of TXA2 while increasing that of PG12,3,44 this could be of considerable therapeutic importance.

Acknowledgments

Prostaglandin standards were gifts from Dr. John Pike (Upjohn Company, Kalamazoo, Michigan). We thank Professor Colin Dollery for permission to study his patients and for his encouragement and Jeremy Beacham for the creatinine and electrolyte determinations.

References


**KEY WORDS** • thromboxane • essential hypertension • prostacyclin
Prostacyclin and thromboxane biosynthesis in mild essential hypertension.
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Hypertension. 1990;15:469-474
doi: 10.1161/01.HYP.15.5.469

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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World Wide Web at:
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