Prostacyclin (PGI₂) Synthesis in the Vascular Wall of Rats with Bilateral Renal Artery Stenosis

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SUMMARY The ability of vessels (rings of arteries and vena cava) to synthesize prostacyclin (PGI₂) "in vitro" was analyzed in the initial (6-day) and chronic (6-week) phase of two-kidney, two clip hypertension. Male Wistar CHbb THOM rats were used. Tissues were incubated for two hours in Krebs solution containing ¹⁴C-arachidonic acid as exogenous substrate. Specimens (in benzene-ethanol 4:1 vol/vol) and the unlabeled standard solutions of arachidonic acid, 6-keto PGF₁₀, PGF₂₀, and PGE₂₀ were spotted on silica gel-G plates for thin layer chromatography. Conversion of ¹⁴C-arachidonic acid to stable metabolite 6-keto PGE₁₀ was used as an index of PGI₂ synthesis. Results shown: 1) PGI₂ is the major PG synthesized by the rat artery wall; 2) PGI₂ synthesis was increased 2.4 times in the initial 6-day period of development of renovascular hypertension (RH); 3) no changes in PGI₂ production were observed in arteries during the chronic 6-week period of RH; 4) abdominal vena cava has little or no capacity to produce PGI₂. As PGI₂ is a potent vasodilator, higher production by arteries during the 6-day period suggests that prostacyclin could play a modulator role on peripheral resistance during the initial phase of renal hypertension. (Hypertension 5 (supp V): V-38-V-42, 1983)

KEY WORDS • prostaglandins • blood vessels • renovascular hypertension

Structural changes of the vessel wall certainly contribute to increased peripheral vascular resistance (PVR) in hypertensive disease,¹ but vasoconstriction must be present for high blood pressure (BP) levels to develop. Thus, in the hypertensive process, factors that regulate contraction of vascular smooth muscle are of particular interest. Prostaglandin I₁ (PGI₁) has been recognized as a potent vasodilator and inhibitor of platelet aggregation²³ and is the principal metabolite of the arachidonic acid or prostaglandin endoperoxides in a number of blood vessels of several animal species and humans.⁶⁻¹¹ The enzyme that synthesizes PGI₁ is almost exclusively concentrated in the endothelial lining of the vessels,⁷⁻¹² but it has been reported to appear, in small proportion, in other vascular layers.⁷⁻¹² Like other prostaglandins, PGI₁ synthesized intramurally can modify vascular smooth muscle tension directly¹⁶⁻¹⁸ or indirectly, altering either the response to diverse vasoactive substances¹⁹⁻²¹ or the adrenergic neurotransmitter release from sympathetic nerve endings.²² This paper deals with the ability of vessels (arteries and vena cava) obtained from rats with bilateral renal artery stenosis to synthesize PGI₁ "in vitro." As far as we know, there is no previous information on this subject in this particular model of renovascular hypertensive animals. Both, the initial (6-day) and chronic (6-week) periods of hypertension were analyzed. Present results show higher PGI₁ synthesis only in arteries after 6 days of increasing BP; the fact suggests that prostacyclin could act as a modulator of PVR in the initial phase of renovascular hypertension (RH); furthermore, because of the absence of demonstrable changes in vessels of 6-week hypertensive rats, the possible role of PGI₁ during the chronic period remains undefined.

**Methods**

The experiments were carried out on male Wistar CHbb THOM rats. High BP was elicited by bilateral stenosis of the renal arteries. Both the acute phase (6-day period) and the chronic phase (6-week period) of the hypertensive process were analyzed. In each study,
the animals were divided into three groups: Intacts, Shams, and Clips. Intacts were rats without any experimental maneuver that were matched in weight; there were 24 rats weighing 250 g that were studied for a 6-day period and 15 rats weighing 370 g studied for a 6-week period. Shams had clips placed on the renal arteries but immediately removed; there were 33 rats for the 6-day period and 14 rats for the 6-week period. Clips had one solid silver clip 0.25 mm of lumen placed on each renal artery; there were 22 rats for the 6-day period and 20 rats for the 6-week period. BP was indirectly recorded by a tail pulse pneumatic transducer connected to an oscilloscope. On the following day, the animals were sacrificed by decapitation, and blood samples were obtained for creatinine measurements (cinetic-colorimetric method). The aorta (the arch excluded) plus iliac arteries and the abdominal vena cava were removed and cleaned of periadventitial tissue. The vascular segments were sliced to 1 mm thickness, and the vessels from several rats were usually pooled (two or three in the case of arteries and three or four in the case of veins). The tissues were prepared in cold temperature (0°C to 4°C) and then kept wet in 1.5 ml of cold Krebs solution until incubation took place. After 30 minutes at 37°C, the vascular segments were transferred to other tubes containing 0.2 μCi 14C-arachidonic acid (New England Nuclear Corporation, Boston, Massachusetts, specific activity = 54.5 mCi/mmol) in 1.5 ml of Krebs solution. Incubation was carried out for 2 hours, with shaking at 37°C. This period of time seems to be adequate, as has been shown by Limas et al.10 The reaction was stopped by acidifying to pH 2.5 to 3 with 0.5 M citric acid. The reaction medium was extracted twice with 4 ml ethyl acetate, and the combined extracts were dried under nitrogen. Then they were taken up in 100 μl of benzene-ethanol (4:1 vol/vol) for thin layer chromatography on silica gel (G type 60, Merck Sharp & Dohme) plates. Standard solutions of arachidonic acid, 6-keto PGF1α, PGE2, and PGF2α were spotted on the same plate of specimens. The chromatographic solvent was the upper phase of ethyl acetate:isooctane:acetic acid:water (110:50:20:100, by volume), and the plates were developed to 15 cm from origin. After drying, the standards were visualized with phosphomolibdic acid (ethanolic solution, 15% p/v), and the corresponding radioactive spots were identified by autoradiography and scrapped off for radioactivity measurement. Conversion of 14C-arachidonic acid to 6-keto PGF1α, the stable metabolite of PG12, was used as an index of vascular PG12 synthesis. To test the inhibition of PG12 synthesis, tranlycypromine (Sigma Chemical Company, St. Louis, Missouri) 500, 1000, and 1500 μg/ml was added to the incubation medium of three samples from nine intact rats, 5 minutes before 14C-arachidonic acid was incorporated. Proteins were measured by the Lowry method.

Data were expressed as means ± se. Statistical significance of the differences were determined either by the two-tailed Student's t test or by one-way analysis of variance and the Newman-Keuls test.

Results

Development of Hypertension

Mean BP values in Shams were: in the 6-day group, 107 ± 2 mm Hg (range 90 to 115) and, in the 6-week group, 121 ± 3 mm Hg (range 100 to 135). Clip rats in which the BP was 10 mm Hg above the highest value of the Sham group were considered hypertensive. Therefore, two Clip rats of the 6-day group (BP under 125 mm Hg) and two of the 6-week group (BP under 145 mm Hg) were discarded. Mean BP values in Clip rats were: in the 6-day group, 147 ± 4 mm Hg (range 125 to 180) and in the 6-week group, 178 ± 5 mm Hg (range 145 to 200). Differences between Shams and Clips were statistically significant both in the 6-day period (t test, p < 0.001) and in the 6-week period (t test, p < 0.001).

Synthesis of Prostaglandins by Arteries

Significant amounts of PG12 (quantified as 6-keto-PGF1α, metabolite) were always present in samples of artery ring incubates (fig. 1). As pointed out in the Methods section, arteries from two to three rats were pooled per sample to be incubated. The BP values were individually recorded, however. This experimental design accounts for the apparent discrepancies in the number of cases in the Sham and Clip groups expressed in Methods and figure 1. In the 6-day period, arteries from hypertensive rats synthesize 2.4 times more PG12 than arteries from Sham rats (p < 0.01). In the 6-week period, there were no significant differences among the groups. Shams of the 6-week period were statistically different from Shams of the 6-day period (p < 0.02).

![Figure 1. Biosynthesis of PGI2 is expressed as 6-keto-PGF1α (6KF1α in the figure). Columns represent mean values, and bars represent the standard errors. In parentheses are the number of experimental cases. In each sample, vessels from two or three rats were incubated. In the 6-day period, data were analyzed by one-way analysis of variance and the Newman-Keuls test. Asterisk (*) indicates a significant difference: Clips vs Intacts, p < 0.05. Clips vs Shams, p < 0.01. In the 6-week period, the asterisk (*) indicates the statistical significance of the difference determined by Student's t test: Shams (6-weeks) vs Shams (6-days) p < 0.02.](image-url)
Identification of the compound as 6-keto-PGF$_{1a}$ was obtained from the following lines of evidences: 1) the compound migrated as unlabeled standards of 6-keto-PGF$_{1a}$ on thin layer chromatography. Moreover, this substance was shown to be more polar than PGF$_{2a}$ and PGE$_2$, as has been reported by other investigators$^{33, 24}$ for 6-keto-PGF$_{1a}$. (In our results, RF = 0.23 as compared with 0.29 for PGF$_{2a}$ and 0.35 for PGE$_2$.) 2) Quantitatively, this compound was the principal product of the PG cascade obtained by incubation of the artery wall, as stated in previous reports.$^7, 8, 12, 23$ 3) Preincubation of the samples with tranylcypromine largely decreased radioactivity (50% to 55% of control) in the spots ascribed to be 6-keto-PGF$_{1a}$, even when the lowest dose (500 µg/ml) was used.

In addition to the spot of 6-keto-PGF$_{1a}$, the presence of other less polar substances was observed in autoradiographs of thin layer chromatography plates of some samples (figure 2). Two of the substances migrated almost similarly to the nonlabeled PGF$_{2a}$ and PGE$_2$ standards; when clearly identified, the correspondent spots were separately scrapped off and quantified. Results (in pmol/100 mg protein) were: in the 6-day period, Intacts (n = 3) PGF$_{2a}$ 149 ± 47, PGE$_2$ 252 ± 100; PGF$_{2a}$ and PGE$_2$ in Shams and Clips were indetectable. In the 6-week period, Intacts (n = 6) PGF$_{2a}$ 241 ± 25, PGE$_2$ 268 ± 31; Shams (n = 1) PGF$_{2a}$ 110; PGE$_2$ 131; Clips (n = 2) PGF$_{2a}$ 148; PGE$_2$ 170.

Synthesis of Prostaglandins by Veins

Rings of abdominal vena cava from either Intact, Sham, or Clip rats of the 6-day period had a clear low capacity to synthesize PGI$_2$; this assertion is based on the fact that 6-keto-PGF$_{1a}$ was not visualized in the autoradiographs in our experiments (fig. 2). Similarly, PGF$_{2a}$ was not observed. Instead, PGE$_2$ was quantified in the six samples from the vena cava of Sham rats (480 ± 125, pmol/100 mg protein) and in three of seven samples from the vessels of clip rats (344 ± 78 pmol/100 mg protein). Further research is needed for reaching conclusive results regarding PGE$_2$ synthesis in the veins of the 6-day period. In the 6-week period, PGI$_2$ synthesis was intended to be enforced by including more tissue per incubation sample (up to 11 veins were pooled in one case). Unfortunately, the determinations had a bad resolution with thin layer chromatography (possibly due to an excess of adventitial lipids) and, consequently, results of PGI$_2$ synthesis by veins in the chronic groups were unclear and are not included in this paper.

Plasma Creatinine

Plasma creatinine was determined in some animals to test renal function. Data, expressed as mg/100 ml, were: 6-day period. Intacts (n = 9) 0.59 ± 0.02; Shams (n = 6) 0.58 ± 0.03; Clips (n = 10) 0.70 ± 0.04; 6-week period. Intacts (n = 20) 0.58 ± 0.02; Shams (n = 4) 0.61 ± 0.04; Clips (n = 6) 0.75 ± 0.05. Differences among groups were not statistically significant (one-way analysis of variance). We have previously reported$^{23}$ plasma creatinine values at the 1st, 2nd, 4th, and 10th week in two-kidney, two clip hypertensive rats when no significant differences between hypertensive and control animals were observed.

Discussion

In this study, 6-keto-PGF$_{1a}$ was actually the major labeled product separated from the artery incubation medium. This finding is in keeping with a series of recent papers that analyzed prostaglandin (PG) synthesis by the entire vessel wall.$^7, 11, 24$ The authors have shown that the stable nonenzymatic metabolite of PGI$_2$ may quantitatively reflect its intramural synthesis, at
least under experimental "in vitro" conditions. So far, similar results have been reported in cultured vascular endothelial cells. 7, 14

In addition to prostacyclin, other PGs and metabolites have been reported formed when homogenates instead of intact vascular tissue were investigated. 8, 24-26 Nevertheless, it must be noted that other substances less polar than 6-keto-PGF_1alpha were also observed in some of our samples (13 of 57 analyzed) after incubation of the entire artery wall and separation of the products by thin layer chromatography; similar results were reported by Limas et al. 10 in different strains. Remarkably, arteries from intact rats, in particular, showed that tendency (see Results). When the radioactive spots were clearly distinguished in the autoradiographs, two of such substances were quantified as PGF_2alpha and PGE_2 since they had migrated similarly to nonlabeled standards in the chromatography plates. It is likely that they represent individual differences in the capacity of the artery wall to generate PGs.

On the other hand, 6-keto-PGF_1alpha was not visualized in autoradiographs from thin layer chromatography plates of vena cava incubates; thus, its presence, if any, would have been insignificant. Furthermore, because of interference from the background radioactivity in the runs, its quantization from the plates was considered unreliable. These results are in harmony with those of Skidgel and Printz, 27 also in rats, using homogenates of the same vessel; the authors concluded that the abdominal segment is even less active than the thoracic segment to produce PGI_2. Wong et al. 25, 28 have proposed that veins would contain more 15-hydroxyprostaglandin dehydrogenase than arteries and this enzyme could form 6-keto-PGF_1alpha in addition to the nonenzymatic metabolite 6-keto-PGF_2alpha. However, because of the absence of distinguishable radio-labeled 6-keto-PGF_1alpha in vena cava incubates, we think PGF_2alpha synthesis was clearly insignificant in our experiments. In this regard, an unequal capacity to synthesize prostacyclin of endothelial cells from different origins could be postulated; as a matter of fact, Herman et al. 28 have observed that the amount of 6-keto-PGF_1alpha formed by the peritoneum after incubation with labeled prostaglandin endoperoxide H_2 was about three times higher than the amount formed by sections of the aorta. Nonsignificant synthesis of PGF_2alpha by the wall of the abdominal vena cava suggests that production of intramural prostacyclin is not fundamental to regulate blood dynamics in great veins of rats either under normal conditions or early hypertension. This conclusion must not be considered to apply to all veins or species in general since human endothelial cells from umbilical cord veins, 12, 29 walls of bovine umbilical veins, 30 and colic and gastric human veins 4 have been shown to produce PGF_2alpha.

In summary, results of the present experiments clearly show that rings of arteries from rats after a short period of RH (6-days) synthesized double the amount of PGF_2alpha produced in tissues of Sham rats incubated with [3H]arachidonic acid substrate. As far as data from conductive arteries apply to small resistance vessels, the fact suggests that intramural prostacyclin may participate in regulation of peripheral vascular tone during the initial phase of RH. We have not found previous information on such brief period of hypertension but similar enhancement of PG production has been reported for vessels from other long-term hypertensive models such as genetic salt-induced hypertension 30 or spontaneous hypertension. 31-33 In these cases, it has been postulated that the increment in biosynthesis could represent a compensatory mechanism by which the increase in BP would be attenuated. 10, 32 Nevertheless, our results from 6-week hypertensive rats showed that stable high BP levels were developed without demonstrable changes in artery PGF synthesis. Thus, the adaptive response of blood vessels mediated by a PG mechanism after prolonged RH seems to be ruled out. However, a modulatory role of PGF_2alpha in the chronic 6-week period could not be completely disregarded since differences in sensitivity to other PGs (PGF_2alpha, F_2alpha, A_2, B_2, D_2) have been described for hypertensive vessels. 32, 34 In this connection, the possibility of changes in vascular responses to local PGF_2alpha, circulating PGF_2alpha or even some enzymic metabolite from plasma 36 has to be kept in mind.

Finally, we must add that the actual "in vivo" significance of the present observations remains unclear. As usual, extrapolations from "in vitro" experiments, as intended in the course of this discussion, must be recognized merely as thought-provoking suggestions.

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