Prostaglandins and Cardiovascular Diseases

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Preface

Despite recent advances in the chemistry of prostaglandins, thromboxane, and leukotrienes, many problems remain to be solved before clinical application of these arachidonate-cascade products can be made. Especially in the field of cardiology and angiology, a comprehensive review covering molecular aspects has been needed by both basic and clinical researchers since it is generally accepted that prostacyclin and thromboxane are interlinked with several serious cardiovascular diseases such as ischemic heart and myocardial infarction. Clinical trials of therapeutic applications have already begun using prostanoids themselves, their synthetic derivatives and their biosynthesis inhibitors.

The "International Symposium on Prostaglandins and Cardiovascular Diseases" was held in Nagoya, Japan on November 23–24, 1984, coinciding with the occasion of the "Kyoto Conference on Prostaglandins". Invited specialists provided insight on their own and related fields and, together with the efforts of the secretary of the symposium, Dr. S. Sugiyama and the editing of the Japan Scientific Societies Press, it is our pleasure to make this valuable information available to the scientific world. The reader will find the coverage extensive: "Prostanoids and hypertension", "Enzyme inhibitors of arachidonate cascade", "Clinical trials of prostanoids and their synthetic derivatives", "Platelet aggregations" and "Vascular effect of leukotrienes".

We hope this book will prove and excellent guide for both basic researchers and clinicals as they confront the many problems posed by prostaglandins.

February 4, 1986

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1

Prostacyclin Biosynthesis in Experimental Hypertension: A Marker of a Fundamental Phospholipid Disturbance That May Contribute to Increased Vascular Reactivity

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Hypertension in man and experimental animals is characterized by excessive vasoconstriction of resistance vessels, due, at least partly, to increased reactivity of vascular smooth muscle to constrictor stimuli. Although alterations in vascular geometry undoubtedly play a role in these abnormalities (*1*), increased reactivity to pressor stimuli could also result from a deficiency of endogenous vasodilators. Vasodilator prostanoids (particularly prostacyclin, PGI_2) are produced by the smooth muscle and endothelium of blood vessels (*2*), and could modulate vascular tone (*3, 4*). Indeed, it has been shown that prolonged inhibition of prostanoid synthesis with indomethacin can elevate peripheral resistance and blood pressure, at least in some species (*5*). However, despite intensive study there is no clear evidence that a deficiency of endogenous prostanoids is a cause of essential hypertension in man or of raised blood pressure in experimental animal models. In this review we present evidence for abnormalities in prostacyclin synthesis by vascular tissues in experimental hypertension, and suggest that these abnormalities may be regarded as markers of a more fundamental disturbance of phospholipid metabolism rather than initiating factors in the development of hypertension. Such a disturbance in the phospholipid stores of vascular tissues could be a potent contributing factor to increased vascular reactivity, common to a variety of hypertensive models and to man.

PROSTACYCLIN BIOSYNTHESIS IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR)

Blood vessels of SHR, derived originally from the Okamoto strain, have increased capacity to produce prostacyclin. This conclusion is supported by two

kinds of studies based on the ability of vascular tissues to convert arachidonic acid to prostacyclin, or its stable hydration product 6-oxo-prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$). In the first of these, we (*6*) and others (*7*) showed that more [^{14}C]-arachidonate was converted to [^{14}C]-6-oxo-$PGF_{1\alpha}$ during *in vitro* incubation of aortic strips from SHR, than in similar strips from age-matched, normotensive Wistar-Kyoto (WKY) control rats (Fig. 1). Pace-Asciak and Carrara (*8*) also showed that the production of prostacyclin is increased with the age of SHR, in parallel with the rise of blood pressure, and they suggested that this represented a compensatory mechanism for the increased peripheral resistance. We also showed that the vasodepressor effects of arachidonate were greater and more prolonged in SHR than WKY, whereas the vasodepressor effects of prostacyclin and nitroprusside were not altered in a specific way by the raised blood pressure

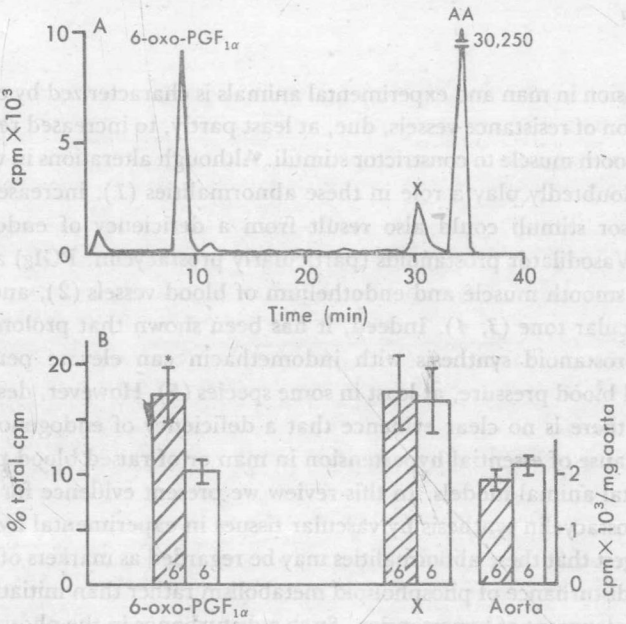

Fig. 1. Conversion of [^{14}C]-arachidonate to prostacyclin in aorta of SHR and WKY control rats. A: HPLC elution profile of the medium obtained from a SHR aorta (see 6 for details of method). The three major peaks correspond to 6-oxo-$PGF_{1\alpha}$ (prostacyclin metabolite), other oxygenated products (X) and unchanged arachidonate (AA). B: mean results from six SHR (▨) and six age-matched WKY (□) rats. Radioactivity in each peak (counts per min, cpm) is expressed as percentage of the total cpm recovered. Radioactivity retained by the aorta was expressed as cpm/mg of aorta. The histograms represent the means and vertical bars the S.E.M.; * indicates a significant difference ($p < 0.05$, Student's *t*-test) between SHR and WKY rats. (Reproduced with permission from Dickens *et al.* (*6*)).

in these rats (*9, 10*). The latter observation suggests that in hypertension there is no specific change in vascular smooth muscle sensitivity to prostacyclin, such as might be induced by changes in prostacyclin receptor number or affinity, nor is there any evidence for a change in mechanisms of prostacyclin disposition.

VASCULAR PROSTACYCLIN IN GOLDBLATT HYPERTENSIVE RATS

We then went on to study the conversion of arachidonic acid to prostacyclin in two commonly used models of renovascular hypertension in rats (*10,11*). In the Goldblatt models renovascular hypertension develops after application of a clip to one renal artery. Although the extent of the blood pressure rise is independent of whether the contralateral kidney is simultaneously removed (1K,1C model) or left intact (2K,1C model), the pathogenetic mechanisms of the resulting one-and two-kidney hypertension are different (*12,13*). Measured in absolute terms, the vasodepressor responses to prostacyclin and nitroprusside correlated well with resting blood pressure in both groups of rats, as was found for SHR and WKY rats (Fig. 2). However, when the responses to standard doses of prostacyclin and nitroprusside were measured as percentages of resting blood pressure, they did not differ significantly between hypertensive rats and the normotensive controls within each group (Fig. 3). In contrast, the vasodepressor effects of arachidonic acid (1–3 mg/kg, i.v.) were much greater in the 1K,1C rats than in their normotensive controls, but did not differ significantly between hypertensive 2K,1C rats and sham operated controls (Fig. 4). In all cases, the effects of arachidonic acid were virtually abolished by indomethacin (10 mg/kg, i.v.). The metabolism of [^{14}C]-arachidonic acid was also studied in isolated aor-

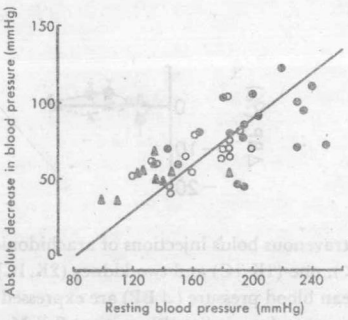

Fig. 2. Relationship between the vasodepressor response to intravenous prostacyclin (PGI_2, 1.0 μg/kg) and the resting level of blood pressure of anaesthetized control (▲), one-(1K,1C) (●), and two-kidney (2K,1C) (○) Goldblatt hypertensive rats. Coefficient of correlation, $r = 0.68$, $p < 0.001$. (Reproduced with permission from Dusting *et al.*(*11*)).

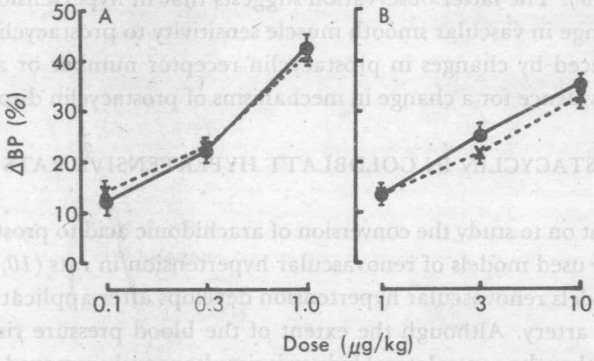

Fig. 3. Vasodepressor responses to intravenous prostacyclin (PGI_2) (A) and nitroprusside (NP) (B) in anaesthetized control (×) and one-kidney (1K,1C) Goldblatt hypertensive rats (●). Results are expressed as the change in blood pressure (Δ BP) measured as a percentage of the resting mean blood pressure, and are plotted as the means ± S.E.M. with 8–12 rats in each group (Reproduced with permission from Dusting *et al.* (*11*)).

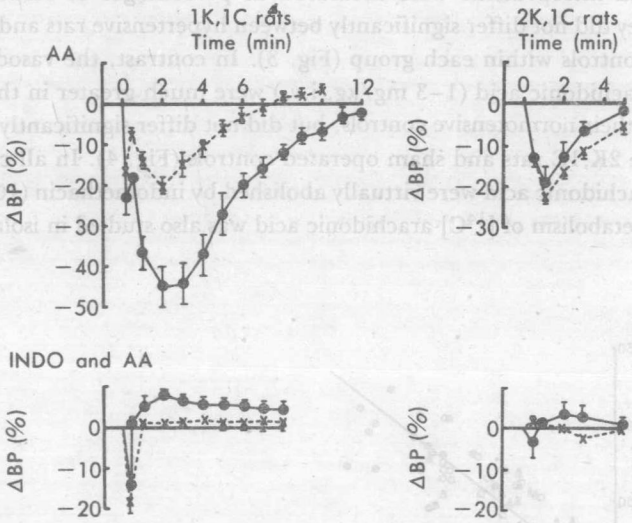

Fig. 4. Vasodepressor responses to intravenous bolus injections of arachidonic acid (AA 3 mg/kg) in anaesthetized control (×), one-(1K,1C) and two-kidney (2K,1C) Goldblatt hypertensive rats (●). Changes in mean blood pressure (Δ BP) are expressed as percentages of the resting mean blood pressure and represent the mean ± S.E.M. with 8–15 rats in each group. The upper panels represent the initial responses and the lower left and right panels show responses to AA in the same rats following intravenous indomethacin (10 mg/kg). The vasodepressor response to arachidonic acid is markedly greater in 1K,1C rats, but not in 2K,1C rats, than their respective controls (Reproduced with permission from Dusting *et al.* (*11*)).

tae of both 1K,1C and 2K,1C rats by high performance liquid chromatography (HPLC) of extracts of the incubation mixture. [^{14}C]-6-oxo-PGF$_{1\alpha}$ was the only prostanoid conversion product recovered from aortic incubations, and significantly more of this metabolite was produced by aortic tissue from 1K,1C rats than from normotensive controls. In contrast, there was no difference in [^{14}C]-6-oxo-PGF$_{1\alpha}$ production between 2K,1C hypertensive rats and controls (Fig. 5). These results therefore demonstrate an enhanced ability of vascular tissue from 1K,1C hypertensive rats to convert exogenous arachidonate to vasodilator prostacyclin, but this is not evident in the two-kidney model. We have no ready explanation for this finding but the result does suggest that the change in arachidonate handling is not secondary to the development of hypertension, but rather represents a mechanism that is common to one-kidney renovascular hypertension and spontaneous hypertension in rats.

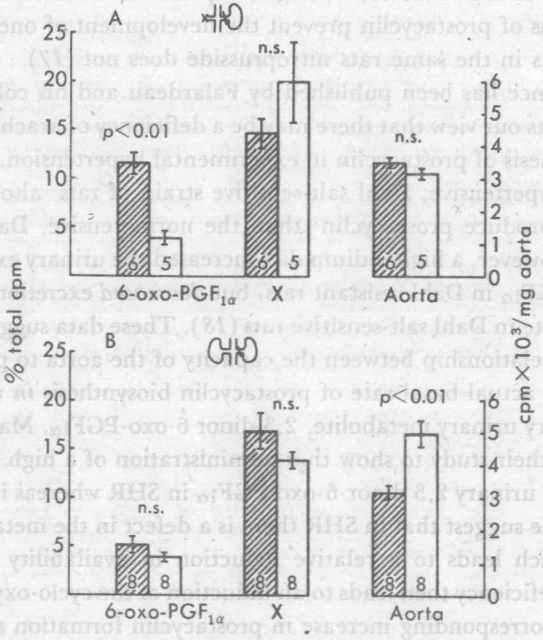

Fig. 5. Conversion of [^{14}C]-arachidonate to prostacyclin product in one-kidney (1K,1C) (A) and two-kidney (2K,1C) (B) Goldblatt hypertensive rats. The mean radioactivities of peaks from the HPLC elution profile were determined as described in Fig. 1. The numbers in the histograms represent the number of aortas used. 6-oxo-PGF$_{1\alpha}$ production was greater in 1K,1C rats (▨) than in their controls (□), but there was no difference between 2K,1C rats (▨) and their controls (□).

DOES ENHANCED PROSTACYCLIN SYNTHESIS HAVE A FUNCTIONAL ROLE IN HYPERTENSION?

Clearly, the conversion of arachidonic acid to prostacyclin is enhanced in blood vessels of both SHR and 1K,1C hypertensive rats. One possible explanation for this finding is that increased vascular prostacyclin formation represents a homeostatic response to the hypertension, presumably as an attempt to lessen peripheral resistance. However, such an explanation seems unlikely because cyclo-oxygenase inhibitors such as aspirin (*14*) or indomethacin (*15*) do not raise blood pressure in the SHR, or in the 1K,1C rat (*16*). This suggests that the demonstrated capacity to produce more prostacyclin from exogenous arachidonate is not accompanied by increased production of prostacyclin from *endogenous* sources of arachidonate. There may, therefore, be a more fundamental defect in arachidonic acid metabolism in these two models, such as a defect in mechanisms that liberate endogenous arachidonic acid from its esterified form in membrane phospholipids, or reduced incorporation of arachidonic acid in these stores (*10,11*). If this were so, it might explain the observation that subdepressor infusions of prostacyclin prevent the development of one-kidney hypertension, whereas in the same rats nitroprusside does not (*17*).

Important evidence has been published by Falardeau and his colleagues (*18,19*) which supports our view that there may be a deficiency of arachidonate available for biosynthesis of prostacyclin in experimental hypertension. Aortas obtained from the hypertensive, Dahl salt-sensitive strain of rats also have a greater capacity to produce prostacyclin than the normotensive, Dahl salt-resistant rats (*20*). However, a high sodium diet increased the urinary excretion of 2,3 dinor-6-oxo-$PGF_{1\alpha}$ in Dahl resistant rats, but *decreased* excretion of this prostacyclin metabolite in Dahl salt-sensitive rats (*18*). These data suggest that there is a reciprocal relationship between the capacity of the aorta to produce prostacyclin and the actual basal rate of prostacyclin biosynthesis *in vivo*, as reflected by its primary urinary metabolite, 2,3-dinor 6-oxo-$PGF_{1\alpha}$. Martineau *et al.* (*19*) extended their study to show that administration of a high sodium diet failed to increase urinary 2,3 dinor-6-oxo-$PGF_{1\alpha}$ in SHR whereas it did in WKY control rats. We suggest that in SHR there is a defect in the metabolism of phospholipids which leads to a relative reduction in availability of free arachidonate. This deficiency then leads to an induction of the cyclo-oxygenase pathway, without a corresponding increase in prostacyclin formation *in vivo*. In the SHR and the Dahl salt-sensitive rat, the defect is probably of genetic origin and may be a significant factor in the pathogenesis of the hypertension: In the 1K,1C rat, the origin of a similar defect is clearly not genetic, but may also be of relevance in the mechanism by which hypertension develops in this model.

OTHER EVIDENCE FOR DISTURBANCES IN PHOSPHOLIPID METABOLISM THAT MAY HAVE A LINK TO HYPERTENSION

Feeding rats for 30 days with a diet deficient in linoleic acid (the dietary essential fatty acid that is a precursor of arachidonic acid in most mammals), together with 0.9% sodium chloride as drinking water, leads to elevation of blood pressure (*21,22*). Subsequent addition of linoleic acid to the diet causes a rapid reversal of the hypertension (*23*). In addition, chronic linoleic acid deficiency leads to induction of the enzymes that transform arachidonic acid into prostaglandins, particularly PGE_2, in renomedullary homogenates (*24–26*). Interestingly, linoleic acid deficiency has also been shown to increase the vasopressor response to angiotensin in pregnant rabbits (*27*). Finally, linoleic acid enriched diets have been reported to reduce the rise in blood pressure in the 1K,1C rat (*28*) and in salt-induced hypertension (*29*). Taken together, these data support our proposition that a deficiency of arachidonate availability may be a contributing factor to the development of hypertension, and, as an epiphenomenon, may lead to a secondary induction of cyclo-oxygenase activity. However, the evidence does not allow us to resolve which arachidonate or phospholipid metabolite is most important for preventing hypertension and modulating vascular reactivity.

To determine whether vascular prostacyclin had a role in the pathogenesis of one-kidney Goldblatt hypertension, we examined the effects of a diet enriched with linoleic acid on the rise in blood pressure and vascular prostacyclin synthesis in this model. Rats were supplied with diets containing 40 energy % safflower oil (of which linoleic acid represented 64% of the fatty acids), 40 energy % coconut oil (mainly saturated fats, containing 7.3% oleic and 2.0% linoleic acid), or standard chow (containing 3.7 energy % linoleic acid). Four weeks after supplying these diets the left renal artery was clipped and the right kidney removed, and the rats were maintained on the diets for a further 4–6 weeks before they were killed and their aortas incubated for measurement of prostacyclin synthesis. Control animals on each diet had a right nephrectomy only. Blood pressure increased faster and to a higher level in 1K,1C rats on normal chow than on either of the high fat diets, although the difference only reached statistical significance (two-way ANOVA (analysis of variance), $F_{1,20} = 6.5$, $p < 0.02$) for the coconut oil group (Fig. 6). The capacity of the aortas to convert [^{14}C]-arachidonate to prostacyclin (see ref. *8* for details of method) was greater in the hypertensive than in the control rats both on the safflower oil diet and on normal diets (Fig. 7). Moreover, total 6-oxo-$PGF_{1\alpha}$ production, measured by radioimmunoassay, was again greater in hypertensive than in control rats, regardless of the diet used (Fig. 7). Therefore, it appears that enriching the diet of hypertensive rats with an excess of the precursor for arachidonate does not