Endogenous biosynthesis of prostacyclin during cardiac catheterization and angiography in man

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ABSTRACT The potent platelet inhibitory and vasodilator properties of prostacyclin suggest that levels of this substance may be of relevance to drug action and pathologic processes in the coronary vascular bed. Attempts to estimate the coronary secretion rate of prostacyclin have relied on measurements of metabolites obtained via cardiac catheter, usually as an adjunct to coronary angiography. To test the hypothesis that such procedures might themselves perturb endogenous biosynthesis of prostacyclin we used mass spectrometry to measure plasma levels of 6-keto-prostaglandin (PG) F$_1$ ($\propto$) across the coronary vascular bed, as well as to assess the excretion of a major urinary metabolite, 2,3-dinor-6-keto-PGF$_1$ ($\propto$) (PGI-M), in patients undergoing cardiac catheterization. PGI-M excretion increased variably from a median 100 to 205 pg/mg creatinine ($p < .01$) during catheterization with angiography and remained elevated 2 to 4 hr after initiation of the procedure. However, cardiac catheterization without angiography also stimulated metabolite excretion, perhaps reflecting catheter-induced vascular trauma. The direct effect of radiocontrast media on vascular release of prostacyclin was indicated by increased PGI-M excretion in healthy volunteers administered intravenous radiocontrast and by studies of the canine coronary artery and jugular vein in vitro. Measurement of plasma 6-keto-PGF$_1$ ($\propto$) after left heart catheterization showed that levels in aortic ($21 \pm 8$ pg/ml) and coronary sinus ($14 \pm 2$ pg/ml) blood were increased compared with peripheral venous levels ($\leq 4 \pm 1$ pg/ml) determined before this procedure. The aortic and coronary sinus concentrations of 6-keto-PGF$_1$ ($\propto$) both increased markedly in one of the five patients after injection of radiocontrast but an aortic coronary sinus gradient of 6-keto-PGF$_1$ ($\propto$) was undetectable before or after angiography. These results indicate that cardiac catheterization and angiography are associated with an increase in prostacyclin formation in vivo.


CONSIDERABLE EVIDENCE implicates platelet activation in the events surrounding vascular occlusion of the coronary circulation. Both autopsy and angiographic investigations have demonstrated that occlusive thrombi usually accompany transmural myocardial infarction,1,2 and several reports suggest that this precedes rather than follows the ischemic episode.3-5 Studies of platelet function in acute myocardial infarction have demonstrated spontaneous platelet aggrega-

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In vitro, abnormal disaggregation of platelets, increased circulating platelet aggregates, and increased levels of platelet secretory granule constituents in peripheral blood.2,6,7 Increased platelet aggregates also have been found in coronary sinus blood during transient ischemic episodes in patients with vasotonic angina.8

More recently, attempts have been made to define the role of oxygenated metabolites of arachidonic acid in the mediation of such occlusive events in vivo. Thromboxane A$_2$, the principle cyclooxygenase product of arachidonic acid in the platelet,9 is a potent vasoconstrictor and stimulus to platelet aggregation, whereas prostacyclin, the predominant product in vascular endothelium,10 has opposite effects on both vascular tone and platelet function. It has been suggested that an “imbalance” in the production rates of these products might be of fundamental importance in the development of thrombotic occlusions of the coronary vascular bed in vivo11 and studies have been performed to obtain biochemical evidence of such a mechanism in
man. Because of their evanescent nature, neither thromboxane A₂ nor prostacyclin can be measured directly in the human circulation. Attempts to apply bioassay techniques ex vivo have proved to be insufficiently accurate for quantitative purposes,¹³ so investigators have generally relied on measurements of stable, inactive metabolites of these compounds, most commonly thromboxane B₂,¹³,¹⁴ and 6-keto-prostaglandin (PG) F₁₀.¹⁵ Levels of these products in coronary sinus and aortic blood have been used to estimate arachidonic acid metabolism in the coronary bed in a variety of occlusive syndromes and to implicate prostacyclin biosynthesis in the mediation of beneficial drug action.¹⁶ In the majority of such studies the samples used have been obtained via cardiac catheter, but if significant prostanoid formation occurs ex vivo or secondary to procedure-related artifacts in vivo, the data provided by this approach are of questionable value in assessing the roles of these compounds in coronary pathophysiology. To test the hypothesis that cardiac catheterization or angiography per se might alter endogenous prostanoid formation in man, we have determined the effects of these procedures on a noninvasive index of prostacyclin biosynthesis in vivo, namely the excretion of 2,3-dinor-6-keto-PGF₁₀ (PGI-M), a major urinary metabolite of prostacyclin in man, and compared the levels of 6-keto-PGF₁₀, the hydration product of prostacyclin, in peripheral plasma with those in the coronary circulation after cardiac catheterization. In addition, we have assessed the possibility of a direct stimulating effect of radiocontrast media on arterial 6-keto-PGF₁₀ formation in vitro.

**Methods**

**Studies in vivo.** Four groups of subjects were studied. Group A consisted of eight men from 38 to 56 years old who underwent left heart catheterization from the femoral approach. The study included left ventricular and coronary angiography with No. 8F Cordis pigtail and Judkins coronary catheters. Venous access was confined to a butterfly needle in a hand vein. Two patients each had one- or two-vessel disease and in the remainder all three vessels were involved by atherosclerosis. Group B consisted of four men, from 48 to 59 years old, who underwent electrophysiologic studies only. The studies involved right heart catheterization only. No medication was administered before any of the studies and anesthesia was confined to infiltration of approximately 6 ml of 2% lidocaine at the site of catheter insertion. Group C comprised seven healthy male volunteers from 27 to 34 years old who received infusions of commonly used radiocontrast dye (meglumine diatrizoate). Group D comprised five male patients from 38 to 56 years old who underwent routine left heart catheterization, with coronary and left ventricular angiography, from the femoral approach. All had given informed consent to the additional placement of a coronary sinus catheter for sampling. All studies on the patients were performed in the Vanderbilt University Hospital Cardiac Catheterization Laboratory, whereas the studies in the normal volunteers were carried out in the Elliot V. Newman Clinical Research Center. The patients and volunteers had abstained from all medications for 24 hr and from aspirin-like drugs for at least 2 weeks before initiation of the study. The protocol was approved by the Vanderbilt University Committee for the Protection of Human Subjects.

In group A, urine was collected for determination of the urinary prostacyclin metabolite (1) for 2 hr before the procedure, (2) for the following 2 hr, including the 45 min period during which the procedure was performed, and (3) for the following 2 hr. The study design was similar for group B, with the exception that the second period lasted 4 rather than 2 hr so that the procedure period of a mean 215 min could be included. In group C (healthy volunteers), 75 ml of meglumine diatrizoate was infused over 5 min. Urine was collected for 2 hr before the infusion and in two sequential 2 hr collections after infusion of contrast. In all cases the patients were asked to void; Foley catheters were not used. The subjects remained supine throughout all the collection periods. The healthy volunteers were conscious of a slight sensation of facial warmth during dosing with intravenous meglumine diatrizoate, but nausea was not induced.

In group D, a peripheral venous sample was first drawn during the initial venipuncture, which consisted of the placement of a 1 inch No. 22G Teflon needle. The right groin and left antecubital fossa were then infiltrated with lidocaine; a No. 8F Judkins left coronary catheter was placed in the descending aorta, and a No. 7F Wilton-Webster coronary sinus catheter was inserted percutaneously through a sheath and advanced to the coronary sinus. Position of the latter was verified by fluoroscopic position and oxygen saturation, but no contrast dye was injected; aortic and coronary sinus samples (10 ml blood each) were drawn contemporaneously as soon as both catheters were in position. A single injection of 8 to 10 ml of the contrast agent was then given, and a second set of aortic and coronary sinus samples were drawn immediately. In patient 1, repositioning of the coronary sinus catheter was necessary after the first left coronary injection and a portion (one-third) of the "coronary sinus sample" was drawn from the right atrium.

Quantitation of levels of PGI-M and 6-keto-PGF₁₀ was accomplished with a previously described stable isotope dilution assay in which negative ion–chemical ionization gas chromatography–mass spectrometry (GC/MS) is used.¹³ Briefly, 5 ng of a deuterated internal standard was added to a 5 ml aliquot of urine. After extraction and back extraction under alkaline and acidic conditions, the sample was derivatized as the methoxime, pentafluorobenzylester. After further purification by thin-layer chromatography, derivatization was completed by formation of the trimethylsilyl ether derivative. Quantitation was accomplished by stable-isotope dilution assay with use of a Hewlett-Packard 5980 instrument operated in the negative ion-chemical ionization mode monitoring mass/charge ratio (m/z) 586 for endogenous PGI-M and m/z 590 for the deuterium-labeled internal standard. Plasma 6-keto-PGF₁₀ was measured by a minor modification of a method previously described.¹⁷ Briefly, plasma samples (5 ml) were spiked with tetradeuterated 6-keto-PGF₁₀ (0.1 ng/ml) and purified by passage over C-18 and silica minicolumns. After derivatization as the methoxime and purification by thin-layer chromatography, the sample was esterified as the pentafluorobenzylester. Derivatization was then completed after a second thin-layer chromatographic procedure by formation of the trimethylsilyl derivative. The samples were then analyzed by stable-isotope dilution assay with use of a Hewlett-Packard 5980 instrument operated in the negative ion-chemical ionization mode, monitoring m/z 614 for endogenous 6-keto-PGF₁₀ and m/z 618 for the tetradeuterated internal standard.

**Plasma β-thromboglobulin.** In group C, in addition to the
Experiments in vitro. Additional studies were performed to define the effects of radiocontrast media on vascular prostacyclin release in vitro. Hearts were obtained from pentobarbital-anesthetized male mongrel dogs that had undergone physiologic studies but had not received any other drugs. The coronary arteries were quickly dissected on ice, placed in ice-cold Krebs-Henseleit bicarbonate buffer, and sliced into 2 mm rings. The rings were rinsed three times with buffer, placed in 25 ml Erlent- meyer flasks (approximately 50 mg tissue per flask) containing 2 ml buffer, and incubated in a Dubnoff shaking metabolic incubator under 95% O₂:5% CO₂ for five 30 min periods at 37°C. The buffer from each period was saved and analyzed for immunoreactive 6-keto-PGF₁α (antibody kindly provided by Dr. R. Workman) by methods previously described.¹⁹ The initial high rate of prostacyclin release in response to tissue manipulation had subsided by the fourth 30 min period. At this time, 2 ml of 25% meglumine diatrizoate in Krebs buffer (or 2 ml buffer alone) was added and the effect on immunoreactive 6-keto-PGF₁α release during the final 30 min period was determined.

Radiocontrast-induced prostacyclin release by the canine jugular vein was examined in a second model system in vitro. In four separate experiments, jugular veins were excised from pentobarbital-anesthetized dogs and immediately placed on a perfusion apparatus. This consisted of proximal and distal canulae between which the veins were interposed. The canulae were housed in a Lucite chamber that permitted circulation of the perfusion medium outside the vein through a separate circuit. Perfusion of the vein at an approximate pressure of 30 mm Hg and at a flow rate of 25 ml/min was accomplished by a peristaltic perfusion pump (Manostat Veristaltic Pump, New York, NY) via silicone rubber tubing. Monitoring of pressure and periodic assessments of flow volume were performed via side ports. Prostacyclin release was estimated from 4 mm (approximately 3 mg tissue) punch biopsy samples obtained from the veins in the following manner. The biopsy sample was immediately placed in a conical plastic beaker containing 10 ml of Ringer’s lactate and bubbled continuously with 95% O₂:5% CO₂ at 37°C for 10 min. The Ringer’s lactate was then discarded and the sample was gently transferred to a second beaker containing the incubation fluid (10 ml) under study. Samples (250 μl) were then withdrawn immediately (zero time) and at 2.5, 5, 7.5, 10, 15, 20, 25, and 30 min and placed on ice for measurement of immunoreactive 6-keto-PGF₁α, as previously described.

Statistical analysis. Data were analyzed by nonparametric methods,¹¹ avoiding assumptions as to the distributions of the variables involved. Data were subjected to one-way analysis of variance by the method of Kruskall and Wallis and subsequent pairwise comparison with control values was by Lord’s U test.

Results

Excretion of PGI-M increased significantly from a median 100 pg/mg creatinine (range 49 to 217) to a median 205 pg/mg creatinine (range 94 to 1102, p < .01) during cardiac catheterization with angiography and remained above control values at a median 165 pg/mg creatinine (range 71 to 774 p < .02) to the time of the final urine collection (figure 1). Although the extent of the increase in PGI-M excretion was variable, it was evident in all of the patients in the study. Metabolic excretion was also increased (p < .01) in patients who underwent cardiac catheterization without concomitant angiography (figure 2). The increment in PGI-M excretion in these patients is not directly comparable to that in those in group A since electrophysiologic studies took longer to complete (average 215 min) than catheterization with angiography (average 45 min). Administration of meglumine diatrizoate into a peripheral vein in healthy volunteers (figure 3) also significantly increased PGI-M excretion from a median 76 pg/mg creatinine (range 45 to 134) to a median 154 pg/mg creatinine (range 82 to 229, p < .01). Plasma levels of β-thromboglobulin failed to increase after meglumine diatrizoate was administered to these subjects (median 16 vs 19 ng/ml; range 12 to 23 vs 13 to 26 ng/ml).

The mean concentration of 6-keto-PGF₁α in periph-

[FIGURE 1. Excretion of PGF-M in patients with coronary artery disease during the 2 hr before cardiac catheterization (Before), the 2 hr from commencement of the procedure (During), which lasted a mean 45 min, and the succeeding 2 hr (After). The median value for each collection is marked by a horizontal bar. Levels of PGF-M rose significantly during the procedure (p < .01) and remained significantly elevated in the final urine collection (p < .05).]
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TABLE 1
Plasma 6-keto-PGF$_{1\alpha}$ (pg/ml)

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Peripheral vein</th>
<th>Before angiography</th>
<th>After angiography</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>50 11</td>
<td>31 21</td>
</tr>
<tr>
<td>2</td>
<td>&lt;2.5</td>
<td>15 20</td>
<td>17 21</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>15 16</td>
<td>21 16</td>
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<tr>
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<td>5</td>
<td>19 16</td>
<td>53 64</td>
</tr>
<tr>
<td>5</td>
<td>&lt;3</td>
<td>5 7</td>
<td>5 6</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>&lt;4 ± 1</td>
<td>21 ± 8$^a$ 14 ± 2$^a$</td>
<td>25 ± 8$^a$ 26 ± 10$^a$</td>
</tr>
</tbody>
</table>

$^a$ p < .01 signifies the difference from peripheral venous values.

Increased in aortic and coronary sinus blood compared with peripheral venous concentrations before cardiac catheterization. Injection of radiocontrast dye further increased plasma levels of 6-keto-PGF$_{1\alpha}$ markedly in one of these patients. One patient (No. 5) in whom cardiac concentrations remained similar to peripheral venous levels admitted to taking a “headache medicine” 48 hr before the procedure. There was no detectable evidence of net prostacyclin production within the coronary bed provided by comparison of aortic and coronary sinus levels of 6-keto-PGF$_{1\alpha}$.

The experiments performed on canine coronary arteries in vitro were consistent with the observations in vivo in suggesting a direct stimulation of vascular prostacyclin release by radiocontrast media (figure 4). The release of 6-keto-PGF$_{1\alpha}$ from coronary artery rings was increased significantly (p < .01) by meglumine diatrizoate when release during a 30 min stimulation period was compared with that during the preceding 30 min baseline period (see Methods).

Three experiments with canine jugular veins were performed. In the first we measured 6-keto-PGF$_{1\alpha}$ output by examination of vein biopsy samples during incubation (1) before perfusion and (2) after perfusion for 20 min with Ringer’s lactate. In the second experiment we assessed prostanoid production by biopsy before incubation in Ringer’s lactate from a vein that had been perfused for 20 min with 20 μl/ml aspirin. This concentration of aspirin was confirmed (19.9 μg/ml) by GC/MS measurement$^{23}$ and exceeds peak plasma levels after a dose (650 mg) of aspirin$^{24}$ that significantly inhibits endogenous biosynthesis of prostacyclin in normal subjects.$^{19}$ In the final experiment we measured 6-keto-PGF$_{1\alpha}$ production in biopsy samples from veins perfused for 20 min with either Ringer’s lactate or meglumine diatrizoate and then incubated in either 25% of the contrast or Ringer’s. The results are summarized in figure 5. Cumulative 6-keto-PGF$_{1\alpha}$ formation was not significantly altered by perfusion with
Ringer's lactate, but perfusion with aspirin (20 μg/ml) inhibited the rise in 6-keto-PGF\textsubscript{1α} concentrations in the incubate with time. Perfusion with meglumine diatrizoate followed by incubation in Ringer's lactate and perfusion with Ringer's lactate followed by incubation in meglumine diatrizoate both dramatically increased the release of 6-keto-PGF\textsubscript{1α} by the biopsy samples.

**Discussion**

In view of the vasoactive and platelet-inhibitory properties of prostacyclin\textsuperscript{10} it is possible that this prostanoïd might play an important role in the prevention of coronary vascular occlusion and in the mediation of drug action in the coronary bed in vivo.\textsuperscript{13-18} In particular, it has been suggested that organic nitrates, which have been reported to enhance prostacyclin release from cultured endothelial cells in vitro,\textsuperscript{25} may act by enhancing endogenous biosynthesis of prostacyclin in vivo. Because of the instability of prostacyclin in biological fluids, attempts to address these questions have relied on assays that assess levels of the inactive metabolites of prostacyclin, most commonly its hydration product, 6-keto-PGF\textsubscript{1α}.\textsuperscript{12} In studies of the coronary circulation, the most commonly analyzed biological fluid has been plasma obtained via cardiac catheters, usually as an adjunctive procedure to cardiac angiography. Experiments using heparin-bonded cardiac catheters indicate that platelet activation during blood withdrawal is likely to seriously confound estimates of thromboxane formation in the coronary circulation based on plasma levels of thromboxane B\textsubscript{2}.\textsuperscript{26} The present investigation demonstrates that mechanical trauma to the endothelium (group B, electrophysiologic stimulus) and radiocontrast injection (group C, normal volunteers), alone or in combination, are capable of stimulating prostacyclin biosynthesis in vivo.

The excretion of PGI-M was increased to a variable extent during cardiac catheterization with angiography in patients with coronary heart disease. One likely cause of this effect is the infusion of a hypertonic medium, radiocontrast dye, during the procedure. We have previously shown that hypertonic media stimulate the release of other prostanoïds from the rat renal papilla\textsuperscript{27} and gastrointestinal tract\textsuperscript{28} and the demonstration that radiocontrast directly enhanced prostacyclin release from the canine coronary artery and jugular veins in vitro. Cumulative release by biopsy samples was not altered by venous perfusion for 20 min with Ringer's lactate (x-x vs o-o). Perfusion for 20 min with 20 μg/ml aspirin abolished the cumulative increase in 6-keto-PGF\textsubscript{1α} (o-o). Both perfusion with 25% meglumine diatrizoate (C-C) and incubation with this medium after perfusion with Ringer's lactate (■■■) evoked a dramatic increase in 6-keto-PGF\textsubscript{1α} release by the vascular samples.
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The tissue of origin of a metabolite detected in urine cannot be definitely identified and it is possible that endothelial stimulation at the site of arterial puncture may have contributed to the increase in PGI-M excretion that we observed. To address this issue more directly we compared plasma concentrations of 6-keto-PGF₁α in peripheral venous blood with that drawn via aortic and coronary sinus catheters. Consistent with the data obtained for PGI-M, plasma 6-keto-PGF₁α concentrations were more than three times higher in the samples obtained via cardiac catheters. An additional marked increment detected after injection of radiocontrast occurred in one of the five patients studied. These data suggest that during routine procedures, catheter trauma represents a more potent stimulus to prostacyclin formation than injection of radiocontrast. No detectable difference was observed between corresponding aortic and coronary sinus levels of 6-keto-PGF₁α, suggesting that procedure-related prostacyclin release is likely to obscure detection of "basal" prostacyclin production across the coronary bed. Furthermore, the levels of 6-keto-PGF₁α that we detected in the coronary circulation by GC/MS were considerably lower than those reported when radioimmunoassay was used (~100 to 200 pg/ml). This suggests that both analytic problems and the procedure-related increment in prostacyclin biosynthesis may complicate the interpretation of data obtained by these methods.

In conclusion, catheter-induced vascular trauma and injection of radiocontrast dye represent independent stimuli to prostacyclin formation in vivo in patients undergoing cardiac catheterization and angiography. Such procedure-related artifacts obscure detection of prostacyclin production within the coronary bed, at least in the absence of a stimulus. Whether paired aortic and coronary sinus sampling would permit detection of net prostacyclin release in response to an administered stimulus remains to be determined by precise physicochemical methods.

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