Prostacyclin, Thromboxane A₂, and Prostaglandin E₂ Formation in Atherosclerotic Human Carotid Artery

Daniel Scott Rush, Morris Daniel Kerstein, John Alexander Bellan, Suzanne Marie Knoop, Philip Rene Mayeux, Albert Lewis Hyman, Philip Jay Kadowitz, and Dennis Benjamin McNamara

Prostaglandin (PG) formation in 16 atherosclerotic human carotid endarterectomy specimens was compared systematically with that of normal carotid artery from seven white pigs and six rhesus monkeys. Prostacyclin (PGI₂) formation (picomoles 6-keto-PGF₁α/2 min/100 μg homogenate protein plus 2 mM glutathione [GSH]) of nonatheromatous intima adjacent proximal (276 ± 32, mean ± SEM) or distal (271 ± 14) to carotid plaque was comparable to that of normal carotid artery from white pig (272 ± 25, NS) and rhesus monkey (219 ± 41, NS), and was greater than stenotic intima (156 ± 17, p < 0.01), subintimal plaque (188 ± 14, p < 0.01), and ulceration (65 ± 16, p < 0.01). GSH modulated PGI₂ synthesis in all carotid specimens except areas of ulceration (p < 0.05), but did not restore PGI₂ formation in atheromatous fractions to basal level. No detectable arterial thromboxane A₂ (TXA₂) formation or GSH-dependent PGE₂ isomerase activity was observed. The decrement in atherosclerotic carotid artery PGI₂ formation was focal (confined to the plaque) and may have been related to loss of effective GSH modulation. These conditions could contribute to a localized imbalance between arterial PGI₂ and platelet TXA₂ with adverse vascular thromboregulatory consequences. (Arteriosclerosis 8:73–78, January/February 1988)

Arterial prostaglandin (PG) synthesis is thought to be important in the maintenance of circulatory homeostasis by contributing to regulation of vascular tone and endothelial platelet adherence. Prostacyclin (PGI₂), the major product of arachidonic acid metabolism in normal vascular endothelium, is a potent vasodilator and inhibitor of platelet aggregation and adherence to the vessel wall.¹ It has been hypothesized that PGI₂ counterbalances the proaggregatory and vasoconstrictive effects of predominantly platelet-synthesized thromboxane A₂ (TXA₂).¹ Recent reports²,³,⁴ suggest that atherosclerotic arteries differ from normal arterial tissues by producing substantially less PGI₂, more PGE₂,⁴ and detectable levels of TXA₂.⁵ Such alterations in PG synthesis could create a localized imbalance between the intravascular actions of PGI₂ and TXA₂, resulting in increased platelet deposition and arterial thrombogenicity at atherosclerotic sites.

The underlying biochemical basis for reported alterations in arterial PG synthesis in atherosclerosis is unclear. Furthermore, it has not been demonstrated whether such changes in atherosclerotic patients are focal (localized only to plaque) or systemic (also found in nonatheromatous arterial tissues). The purpose of this study was to identify enzymatic changes in carotid endarterectomy specimens that might account for these previously observed disturbances of eicosanoid metabolism and investigate the potential of glutathione (GSH), an endogenous antioxidant, to modulate PG synthesis.

Methods

Normal Carotid Artery Specimens

A single specimen of nonatherosclerotic human carotid artery (n = 1) was harvested with appropriate authorization from a brain-dead cadaver kidney donor. Fresh carotid arteries from young adult, white (cross-bred, market grade) pigs (n = 7) and rhesus monkeys (n = 6) were used as control specimens. The animal experiments conformed to principles of animal care established by the Tulane Medical Center Advisory Committee for Animal Resources, "Principles of Laboratory Animal Care," and the "Guide for the Care and Use of Laboratory Animals" (NIH publication No. 80-23, revised 1978).

Atherosclerotic Carotid Artery Specimens

Occlusive atheromatous internal carotid artery plaques were removed from patients (n = 16) by standard carotid endarterectomy with appropriate informed consent. Human specimens were obtained in accordance with guidelines established by the Code of Federal Regulations (46.101,b,5) and with approval of the Tulane Medical Center Committee on Use of Human Subjects.

Carotid plaques were dissected into the following fractions for systematic analysis of eicosanoid formation (Figure 1): 1) atherosclerotic, but nonatheromatous, intima adjacent proximal (n = 10) and distal (n = 7) to the occlusive internal carotid plaque; 2) atherosclerotic stenotic intima overlying plaque in the point of arterial narrowing (n = 9);
**Assay of Prostaglandin Formation**

A general discussion of eicosanoid metabolism, PG synthetic pathways, and the pivotal role of PGH2 (the product of arachidonic acid metabolism by cyclooxygenase, and substrate for the terminal enzymes PGI2 synthase in vascular tissues and TXA2 synthase in platelets) is available from other sources. All of the patients in this study were undertaken.

All vascular specimens were immersed in ice-cold 0.1 M potassium phosphate buffer (pH 7.4) and either assayed immediately or stored at -45°C. No statistical difference (NS) in enzyme activity was observed between specimens assayed immediately and those stored for assay at a later date. At the time of analysis, specimens were divided into fractions, blotted dry, weighed while maintaining a temperature of 0°C to 4°C, minced, and then homogenized in approximately 0.5 mm for comparative purposes.

PGH2 synthesis occurs predominantly in the endothelium lining the arterial intima; accordingly, all fractions with a luminal surface were grossly dissected to a thickness of 100 μl of 0.1 M potassium phosphate buffer. Homogenate protein with 2 mM GSH and was determined over a 2-minute incubation of PGH2 at each homogenate protein concentration with 2 mM GSH. Basal PGI2 formation in proportion to the homogenate protein was determined using both parametric and nonparametric methods with comparable results; p<0.05 was considered statistically significant. One-way analysis of variance with Newman-Keuls post hoc test and the Kruskal-Wallis test were used for group comparisons. Student’s paired t test and Wilcoxon signed rank test were used to determine the effects of GSH.

Data Analysis

PG formation for each specimen is expressed as mean ± standard error of the mean (SEM) in picomoles of stable PG breakdown product formed per 2-minute incubation of PGH2 at various homogenate protein concentrations, with or without 2 mM GSH. Data were analyzed using both parametric and nonparametric methods with comparable results; p<0.05 was considered statistically significant.

**Results**

**Prostacyclin Synthase Activity**

PGI2 synthesis was determined by identification of 6-keto-PGF1α, the only product of spontaneous PGI2 hydrolysis under these incubation conditions. Basal PGI2 synthase activity (picomoles 6-keto-PGF1α formed during 2-minute incubation of PGH2 at each homogenate protein concentration with 2 mM GSH) was determined over a wide range of protein levels and demonstrated increasing PGI2 formation in proportion to the homogenate protein concentration (Figure 2). These data indicate the presence of an active PGI2 synthase in carotid artery specimens. An increase in PGI2 synthase activity was found with the addition of 2 mM GSH at each homogenate protein level (p<0.01, data not shown).

No difference in PGI2 synthase activity was identified at any protein concentration between normal carotid artery from white pigs or rhesus monkeys and nonatheromatous intima adjacent (proximal or distal) to carotid plaque from atherosclerotic patients (Figure 2) (NS). Basal PGI2 formation (picomoles 6-keto-PGF1α/2 min/100 μg homogenate protein with 2 mM GSH) of arteriosclerotic, nonatheromatous, intima adjacent proximal (276 ± 32) and distal (318 ± 52) subintimal plaque (n = 12); and 4) intimal ulceration (n = 4). Not every carotid endarterectomy specimen contained sufficient material from each of these regions for complete determination of PG formation, as noted by the variation in the number of specimens for each fraction. PGI2 synthesis occurs predominantly in the endothelium lining the arterial intima; accordingly, all fractions with a luminal surface were grossly dissected to a thickness of approximately 0.5 mm for comparative purposes. All vascular specimens were immersed in ice-cold 0.1 M potassium phosphate buffer (pH 7.4) and either assayed immediately or stored at -45°C. No statistical difference (NS) in enzyme activity was observed between specimens assayed immediately and those stored for assay at a later date. At the time of analysis, specimens were divided into fractions, blotted dry, weighed while maintaining a temperature of 0°C to 4°C, minced, and then homogenized in ten volumes of cold phosphate buffer. Homogenate protein concentrations were determined using the Bio-Rad assay. Radiolabelled PG endoperoxide H2 (14C-PGH2) was prepared from radiolabelled arachidonic acid in our laboratory using a previously published protocol.

Quantitative radiochromatography was used to study 14C-PGH2 metabolism by the following methodology. The reaction substrate, 10 μM 14C-PGH2 (15,000 cpm), was evaporated to dryness under a stream of nitrogen in a cold (0°C) Brinkman centrifuge tube. The assay was then started by addition of 100 μl of 0.1 M potassium phosphate buffer (pH 7.4) containing varying concentrations of tissue homogenate protein with or without 2 mM GSH and vortexed immediately. After a 2-minute incubation at 37°C, the reaction was stopped and PG products were extracted by addition of 400 μl of an ice-cold solution of ethyl acetate/methanol/0.2 M citric acid (pH 2.0) (15:2:1). The reaction tubes were again vortexed, centrifuged at 12,000 g for 30 seconds, and frozen quickly on dry ice. The upper organic layer (containing 85% to 95% of the 14C-radiolabel) was spotted for thin-layer chromatography (TLC) on Analtech silica gel GHL plates and then developed with the solvent system, ethyl acetate/acetic acid/hexane/water (54:12:25:60, organic phase). PG products were identified by exposure to iodine vapor and comparison with the migration of authentic PG standards on the TLC plate. Radiolabelled PG products were quantitated using a radiochromatogram scanner (Packard 7201B or Technical Associates Hydra HY-3) mated to a computer. Duplicate determinations were performed at each homogenate protein concentration for the various tissue samples. The lowest detectable level of PGI2 product formation by radiochromatic TLC was 1% of substrate conversion (10 picomoles).
(271 ± 14) to the internal carotid plaque was comparable to that of normal carotid from young adult white pigs (272 ± 25) (NS) and rhesus monkeys (219 ± 41) (NS). Data from the single specimen of normal human carotid artery (not entered into the statistical analysis) indicate values of PGI\(_2\) formation also within the range of these specimens.

Figure 3 shows the 6-keto-PGF\(_{1α}\) formation by fractions of human carotid endarterectomy specimens at various homogenate protein concentrations. PGI\(_2\) formation by nonatheromatous intima proximal and distal to carotid plaque was similar at all protein concentrations (NS, data not shown). A decrement in PGI\(_2\) formation was demonstrated between nonatheromatous intima adjacent proximal or distal to the carotid plaque and stenotic intima underlying the plaque, subintimal plaque, and areas of intimal ulceration. It should be noted that PGI\(_2\) formation in subintimal plaque was comparable to stenotic intima underlying plaque at the point of arterial narrowing; the least activity was found in intimal ulcerations. Other than areas of ulceration, all specimens increased PGI\(_2\) formation with the addition of 2 mM GSH (p<0.05) (Table 1).

**Thromboxane A\(_2\) Synthase Activity**

TXA\(_2\) formation determined by identification of TXB\(_2\), the stable product of TXA\(_2\) hydrolysis, was not detected at any protein concentration in homogenates of white pigs, rhesus monkeys, or human carotid artery specimens, and if present was, thus, less than 10 picomoles.

**Glutathione-Dependent Prostaglandin E\(_2\) Isomerase Activity**

Data in Tables 1 and 2 demonstrate an inverse relationship between PGI\(_2\) and PGE\(_2\) formation for all carotid artery specimens in both degree of plaque pathology and homogenate protein concentration. In all cases in which PGI\(_2\) formation was low, PGE\(_2\) formation was high; a reciprocal relationship was observed when PGI\(_2\) formation was high. PGE\(_2\) formation decreased with increasing homogenate protein concentration, although no difference in PGE\(_2\) formation by carotid artery from white pigs, rhesus monkeys, or human specimens was found at any given protein concentration (NS). Additionally, no increase in PGE\(_2\) formation was detected after addition of 2 mM GSH (NS).

**Discussion**

This study demonstrated the presence of an active PGI\(_2\) synthase in carotid artery from white pigs, rhesus monkeys, and in human normal and atherosclerotic tissue. Nonatheromatous arterial intima adjacent to atherosclerotic carotid plaque formed PGI\(_2\) in quantities indistinguishable from that of normal carotid artery specimens from young adult white pigs and rhesus monkeys. In contrast, atheromatous fractions of human carotid plaque formed significantly less PGI\(_2\) than adjacent arteriosclerotic, nonatheromatous, arterial intima and was least in areas of plaque ulceration. Although other authors\(^5\) \(^4\) have reported decreased PGI\(_2\) formation relative to the severity of atherosclerosis, this study was the first to systematically evaluate
the focal nature of decreased PGI₂ formation in complex atherosclerotic carotid specimens.

A number of cells in the arterial wall (endothelium and smooth muscle) are capable of PGI₂ synthesis. Considerable variation in the distribution of cellular elements within advanced human carotid plaques was recently reported.10,11 Our data demonstrate that decreased PGI₂ formation in patients with advanced atherosclerosis is focal (i.e., localized to atheromatous plaque) and not a consequence of generalized arteriosclerosis. The data do not indicate whether decrements in PGI₂ formation observed in atheromatous fractions resulted from a less active PGI₂ synthase, cellular heterogeneity, or dilution of PGI₂ synthase by atheromatous material.

Demonstration of PGI₂ synthesizing capability in human and experimental arteriosclerosis is not without controversy. Diminished PGI₂ formation in atherosclerotic specimens has been reported previously,2,3,4,12 but other studies have found insignificant differences13 or increases.14 The significance of in vitro studies of PGI₂ formation by atherosclerotic arterial specimens has recently been questioned. Quantitative correlation between the in vitro capacity of vascular tissues to synthesize eicosanoids and actual endogenous biosynthetic rates may be tenuous, and discrepancies between the two have been suggested.15 Direct in vivo assay of PGI₂ formation by atherosclerotic arteries is not currently possible and, thus, other authors have investigated endogenous PGI₂ synthesis by indirect methods.15,16 Basal production of PGI₂ under physiologic conditions is reported to be low. Patients with severe atherosclerosis and evidence of platelet activation were observed to excrete higher urinary levels of 2,3-dinor-6-keto-PGF₁α (one of a number of in vivo PGI₂ metabolites) than normal subjects;15 these authors concluded that reduced PGI₂ formation by atherosclerotic specimens observed in vitro was, therefore, physiologically irrelevant. It was proposed that endogenous production of PGI₂ was lower than normal vascular tissues are capable of producing because of the lack of a stimulus and, conversely, higher in atherosclerotic patients. Possible mechanisms for stimulation of PGI₂ formation (increased platelet/vascular interactions) are speculative.15

Increased urinary excretion of a PGF₂α metabolite from unknown tissue origin in atherosclerotic patients and studies indicating a localized reduction in PGI₂ synthesis by atheromatous plaques are not necessarily contradictory. Whether reported in vivo increases in PGI₂ formation in patients with advanced atherosclerosis occur in atheromatous or nonatheromatous arterial regions is currently unknown and cannot be determined conclusively by indirect assay methods. If, as suggested by urinary PGF₂α metabolite excretion studies, increased PGI₂ is produced in atherosclerotic patients, the site of PGI₂ formation may not be from plaques themselves, but from neighboring nonatheromatous arterial intima. Definitive conclusions regarding endogenous vascular PGI₂ biosynthesis in atherosclerotic patients, based on a quantitative correlation with urinary eicosanoid metabolite levels, may be premature.14

Deficiencies in PGI₂ synthase activity may set limits on endogenous PGI₂ biosynthesis by atheromatous plaques.

### Table 1. Effect of Reduced Glutathione on Prostaglandin Endoperoxide H₂ Metabolism by Fractions of Atherosclerotic Human Carotid Artery

<table>
<thead>
<tr>
<th>Specimen (no.)</th>
<th>25 μG protein</th>
<th>100 μG protein</th>
<th>200 μG protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 GSH</td>
<td>2 mM GSH</td>
<td>0 GSH</td>
</tr>
<tr>
<td>Pig (7)</td>
<td>219 ± 22</td>
<td>188 ± 16</td>
<td>141 ± 11</td>
</tr>
<tr>
<td>Monkey (6)</td>
<td>207 ± 23</td>
<td>209 ± 23</td>
<td>157 ± 22</td>
</tr>
<tr>
<td>Human* (10)</td>
<td>224 ± 18</td>
<td>240 ± 26</td>
<td>145 ± 11</td>
</tr>
<tr>
<td>Human† (1)</td>
<td>338 ± 28</td>
<td>280</td>
<td>180</td>
</tr>
</tbody>
</table>

The results are expressed as the means ± SEM of picomoles of PGH₂ product/2-minute incubation of 10 μM PGH₂/0.1 ml homogeneate protein, with and without 2 mM GSH.

6-keto-PGF₁α = stable breakdown product of prostacyclin (PGI₂) in vitro; PGE₂ = prostaglandin E₂; GSH = glutathione.

*Nonatheromatous intimal fraction of human carotid artery adjacent proximal to carotid plaque. †Normal human carotid from brain-dead cadaver kidney donor (for reference only, not included in statistical analysis).

### Table 2. Prostaglandin E₂ Formation in Carotid Artery

<table>
<thead>
<tr>
<th>Specimen (no.)</th>
<th>25 μG protein</th>
<th>100 μG protein</th>
<th>200 μG protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 GSH</td>
<td>2 mM GSH</td>
<td>0 GSH</td>
</tr>
<tr>
<td>Pig (7)</td>
<td>219 ± 22</td>
<td>188 ± 16</td>
<td>141 ± 11</td>
</tr>
<tr>
<td>Monkey (6)</td>
<td>207 ± 23</td>
<td>209 ± 23</td>
<td>157 ± 22</td>
</tr>
<tr>
<td>Human* (10)</td>
<td>224 ± 18</td>
<td>240 ± 26</td>
<td>145 ± 11</td>
</tr>
<tr>
<td>Human† (1)</td>
<td>338 ± 28</td>
<td>280</td>
<td>180</td>
</tr>
</tbody>
</table>

The results are expressed as the means ± SEM of picomoles of PGE₂/2-minute incubation of 10 μM PGH₂ homogeneate protein concentration, with and without 2 mM glutathione (GSH).

*Nonatheromatous intimal fraction of human carotid artery adjacent proximal to carotid plaque. †Normal human carotid from brain-dead cadaver kidney donor (for reference only, not included in statistical analysis).
regardless of the level of stimulation. Although the theory is speculative and requires further experimentation, the nonatheromatous portions of the arterial system may be subjected to increased traumatic or chemically mediated platelet/vascular interactions by proximity to neighboring plaques and, thus, stimulated to produce PGI₂. Additionally, hemodynamic factors such as increased flow velocity or wall shear stress in nonatheromatous regions adjacent to atherosclerotic arterial stenoses could stimulate PGI₂ formation. Whether PGI₂ synthesis by nonatheromatous intima can regulate platelet adherence to nearby plaques is currently unknown. Flow separation and other complex fluid disturbances at the carotid bifurcation and in the proximity of occlusive plaques may interfere with the local dispersion of PGI₂ in the blood stream, precluding inhibition of platelet aggregation and adherence. It has been shown in vitro that small concentrations of PGI₂ prevent thrombus formation, but larger concentrations are needed to reduce platelet adhesion. Imbalances between PGI₂ and TXA₂ could have localized adverse thromboregulatory consequences in the immediate environment of atherosclerotic plaques, not necessarily reflected in circulating or urinary PGI₂ metabolites.

It has been proposed that lipid peroxides or other reactive oxygen species found in atherosclerotic vessels inhibit PGI₂ formation. PGI₂ synthase is uniquely sensitive to oxidant (O₂⁻) released after peroxidation of 15-hydroperoxy-eicosatetraenoic acid (15-HPETE), a lipoxygenase product of arachidonic acid found in atherosclerotic plaques. A recent study from our laboratory found that PGI₂ formation in coronary artery microsomes could be modulated in a dose-dependent manner by GSH. We suggested that PGI₂ synthase activity determinations with the addition of GSH demonstrate substantially less PGI₂ formation when PGH₂ is enzymatically by GSH-dependent PGE₂ isomerase activity in carotid artery formed more PGI₂ in the presence of GSH; however, GSH was unable to restore PGI₂ formation in carotid plaque to a level comparable to that of the surrounding nonatheromatous intima. These data suggest that a reduction in, or loss of, the effectiveness of intracellular GSH to maintain basal PGI₂ synthase activity as a consequence of oxidative or other mechanisms could contribute to diminished PGI₂ formation.

TXA₂ synthesis by arterial tissues is controversial, but TXA₂ formation by whole atherosclerotic human carotid plaque specimens has been recently reported. We found no detectable TXA₂ formation in any normal or atherosclerotic carotid artery homogenates. Because of the restricted quantity of material in each carotid plaque specimen, it is possible that homogenate protein concentrations used in this study were too low to support detectable levels (10 picomoles) of TXA₂ formation by radiometric TLC. Nevertheless, current data suggest that neither normal nor atherosclerotic carotid artery have substantial capability to form TXA₂. Arterial TXA₂ formation is, thus, unlikely to be a major contributing factor in platelet adherence to carotid plaque.

The reciprocal relationship between PGI₂ and PGE₂ formation has been observed previously in atherosclerotic human aorta. It was proposed that atherosclerotic aorta shifts eicosanoid metabolism from PGI₂ synthesis to produce PGE₂ predominantly. PGE₂ formation can occur by spontaneous hydrolysis of PGH₂ or enzymatically by GSH-dependent PGE₂ isomerase of PGH₂. Our data demonstrate substantially less PGE₂ formation when PGH₂ is converted rapidly to PGI₂ by PGI₂ synthase, and vice versa; additionally, no GSH-dependent PGE₂ formation was observed. We conclude that in carotid artery homogenates most, if not all, PGE₂ was formed nonenzymatically secondary to spontaneous hydrolysis of PGH₂. The significance of this apparent lack of GSH-dependent PGE₂ isomerase in carotid artery is currently unknown but could preclude substantial endogenous PGE₂ formation. PGE₂ reportedly promotes the second phase of platelet aggregation. While it is doubtful that unregulated PGE₂ formation by spontaneous hydrolysis of PGH₂ contributes to carotid arterial thromboregulatory mechanisms, PGE₂ formed nonenzymatically could oppose platelet inhibitory effects of PGI₂. Previous work in our laboratory, which unmasked the presence of GSH-dependent PGE₂ isomerase activity in bovine coronary artery microsomes, demonstrated the need for appropriately controlled experiments before the presence of GSH-dependent PGE₂ isomerase in vascular tissues could be definitely excluded.

Acknowledgments

The authors thank the following who also contributed to this study: Lisa Chauvin for manuscript preparation; Betsy Ewing for the illustrations; Gae O. Decker-Garrad for editorial assistance; Dr. Robert Wolf and Gary Baskin, Delta Regional Primate Research Center; and Dr. Janet Rice, Department of Biostatistics and Epidemiology, Tulane University School of Public Health and Tropical Medicine.

References

4. Rolland PH, Jouve R, Pellegrih E, Mercer C, Serradimign

Index Terms: prostacyclin • thromboxane A₂ • prostaglandin E₂ • atherosclerosis • human • carotid artery • pig • monkey
Prostacyclin, thromboxane A2, and prostaglandin E2 formation in atherosclerotic human carotid artery.
D S Rush, M D Kerstein, J A Bellan, S M Knoop, P R Mayeux, A L Hyman, P J Kadowitz and D B McNamara

Arterioscler Thromb Vasc Biol. 1988;8:73-78
doi: 10.1161/01.ATV.8.1.73
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1988 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/8/1/73

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org/subscriptions/