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### Research Article

Platelets adhere to the subendothelial layer of newly deendothelialized arteries. Attachment can be reduced with exogenous prostacyclin (PGI<sub>2</sub>). Thus, the subendothelium may be unable to produce sufficient PGI<sub>2</sub> to prevent platelet adherence and subsequent platelet-platelet interaction. Consistent with this explanation are data from an earlier report (1977. Moncada S., A. G. Herman, E. A. Higgs, and J. R. Vane. *Thromb. Res.* 11:323-344) indicating that the smooth muscle layer of aorta has only 10-15% of the capacity of endothelial cells to synthesize PGI<sub>2</sub>. We have measured the concentrations of PGI<sub>2</sub> synthase and prostaglandin endoperoxide (PGH) synthase in bovine aorta and obtained results quite different from those described in this earlier report. Tandem immunoradiometric assays for PGI<sub>2</sub> synthase and PGH synthase antigens were used to quantitate these proteins in detergent-solubilized homogenates of endothelial cells and smooth muscle tissue prepared from 10 different bovine aorta. The concentrations of PGI<sub>2</sub> synthase in endothelial cells and smooth muscle were found to be the same. However, the concentration of PGH synthase in endothelial cells averaged greater than 20 times that of smooth muscle. Results similar to those determined by immunoradiometric assay were also obtained when PGH synthase and PGI<sub>2</sub> synthase catalytic activities were measured in preparations of endothelial and smooth muscle cells. Furthermore, when bovine aorta and renal arteries were subjected to immunocytofluorescence staining using monoclonal [...]

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# Concentrations of Prostaglandin Endoperoxide Synthase and Prostaglandin I<sub>2</sub> Synthase in the Endothelium and Smooth Muscle of Bovine Aorta

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**ABSTRACT** Platelets adhere to the subendothelial layer of newly deendothelialized arteries. Attachment can be reduced with exogenous prostacyclin (PGI<sub>2</sub>). Thus, the subendothelium may be unable to produce sufficient PGI<sub>2</sub> to prevent platelet adherence and subsequent platelet-platelet interaction. Consistent with this explanation are data from an earlier report (1977. Moncada S., A. G. Herman, E. A. Higgs, and J. R. Vane. *Thromb. Res.* 11:323-344) indicating that the smooth muscle layer of aorta has only 10-15% of the capacity of endothelial cells to synthesize PGI<sub>2</sub>. We have measured the concentrations of PGI<sub>2</sub> synthase and prostaglandin endoperoxide (PGH) synthase in bovine aorta and obtained results quite different from those described in this earlier report. Tandem immunoradiometric assays for PGI<sub>2</sub> synthase and PGH synthase antigens were used to quantitate these proteins in detergent-solubilized homogenates of endothelial cells and smooth muscle tissue prepared from 10 different bovine aorta. The concentrations of PGI<sub>2</sub> synthase in endothelial cells and smooth muscle were found to be the same. However, the concentration of PGH synthase in endothelial cells averaged >20 times that of smooth muscle. Results similar to those determined by immunoradiometric assay were also obtained when PGH synthase and PGI<sub>2</sub> synthase catalytic activities were measured in preparations of endothelial and smooth muscle cells. Furthermore, when bovine aorta and renal arteries were subjected to immunocytofluorescence staining using monoclonal antibodies to PGI<sub>2</sub> synthase,

fluorescence staining of equivalent intensity was detected in both the endothelial cells and the smooth muscle. Moreover, the intensity of fluorescence was similar throughout cross-sections of vascular smooth muscle, indicating that there is no gradient in PGI<sub>2</sub> synthase concentrations between the endothelium and adventitia. Our results indicate that the propensity of platelets to adhere to the subendothelium of deendothelialized arteries and form aggregates cannot be attributed simply to an inability of the denuded vasculature to produce PGI<sub>2</sub> from PGH<sub>2</sub>, but may be a consequence of the low PGH synthase activity of smooth muscle. Consistent with this concept are the results of Eldor et al. (1981. *J. Clin. Invest.* 67:735-741) who reported that increases in PGH synthase activity are associated with formation of a nonthrombogenic neointima.

## INTRODUCTION

Prostaglandin I<sub>2</sub> (prostacyclin, PGI<sub>2</sub>)<sup>1</sup> is a potent vasodilator and antithrombogenic agent (1-3) formed from the prostaglandin endoperoxide PGH<sub>2</sub> through the action of prostacyclin synthase (4-6). Early studies on rabbit aorta indicated that the endothelium was the major site of vascular PGI<sub>2</sub> synthesis and that smooth muscle had considerably less prostacyclin synthase activity than endothelial cells (7). These results formed the basis for the hypothesis that adherence of platelets to the vascular subendothelium resulted from a relative inability of vascular smooth muscle to synthesize

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<sup>1</sup> *Abbreviations used in this paper:* BAE cells, bovine aortic endothelial cells; DMEM, Dulbecco's-modified Eagle's medium; IRA, immunoradiometric assay; PGH synthase, prostaglandin endoperoxide synthase; PGI<sub>2</sub> synthase, prostaglandin I<sub>2</sub> (prostacyclin) synthase.

PGI<sub>2</sub> from PGH<sub>2</sub> (2, 7). It is now clear, however, that arterial smooth muscle cells (8–11) cultured from a variety of sources are capable of synthesizing PGI<sub>2</sub> from PGH<sub>2</sub> at rates comparable to those obtained with cultured endothelial cells (10, 11).

We recently prepared monoclonal antibodies to PGI<sub>2</sub> synthase (6). In using these reagents to determine the subcellular location of this enzyme by immunocytochemistry (12), we noted that the intensity of PGI<sub>2</sub> synthase-positive immunofluorescence was similar in both the endothelial cells and the underlying smooth muscle. This observation, coupled with previous evidence for efficient PGI<sub>2</sub> synthesis by cultured smooth muscle cells (8–11), led us to quantitate PGI<sub>2</sub> synthase in fresh vascular smooth muscle and endothelial cell extracts using a newly developed immunoradiometric assay (IRA) for this protein. In contrast to the results obtained by Moncada et al. (7), our data indicate that the concentration of PGI<sub>2</sub> synthase is the same in different layers of the vasculature; however, the results of parallel studies quantitating PGH synthase indicate that this enzyme is present in considerably higher concentrations in endothelial cells than in smooth muscle.

## METHODS

**Materials.** Diethyldithiocarbamate and Tween 20 were obtained from Sigma Chemical Co. (St. Louis, MO). Dulbecco's-modified Eagle's medium (DMEM), antibiotic-antimycotic (100×), and fetal bovine serum were all purchased from Gibco Laboratories (Grand Island, NY). Collagenase (CLS II) was from Worthington Biochemical Corp. (Freehold, NJ). [5,6,8,9,11,12,14,15-<sup>3</sup>H]Arachidonic acid (100 Ci/mmol) and Na<sup>125</sup>I (100 mCi/ml) were products of New England Nuclear (Boston, MA). Triton X-100 and rabbit anti-human Factor VIII serum were from Calbiochem-Behring Corp., American Hoechst Corp. (San Diego, CA). Flurbiprofen was generously supplied by Dr. Udo Axen of the Upjohn Co. (Kalamazoo, MI). Fluorescein isothiocyanate-labeled rabbit anti-mouse IgG was from Miles Laboratories, Inc., Research Products Div. (Elkhart, IN).

**Isolation of endothelial cells.** Endothelial cells were isolated from bovine abdominal aorta using a procedure similar to the one described by Macarak et al. (13). Aortae were obtained fresh at slaughter. The adventitia was removed and the tissue was immersed in DMEM (4°C) for transport to the laboratory (~30 min). Collateral arteries were tied with suture and the bottom of the aorta clamped with a hemostat. The lumen was washed with 3 vol of Krebs buffer (composition, in mM: 118 NaCl, 25 NaHCO<sub>3</sub>, 14 glucose, 4.7 KCl, 2.5 CaCl<sub>2</sub>, 1.8 MgSO<sub>4</sub>, and 1.8 KH<sub>2</sub>PO<sub>4</sub>), pH 7.4 then filled with 0.1% collagenase (CLS II) in Krebs buffer, pH 7.4. The aortae were then allowed to hang for 30 min at 37°C. The collagenase solution was removed, the lumen filled two-thirds full with DMEM and the aorta rotated between fingers and thumb to dislodge the endothelium. The contents of the lumen were collected in polyethylene tubes and centrifuged at 1,000 g for 5 min to collect the bovine aorta endothelial (BAE) cells, which were stored at -80°C until use. For most experiments, BAE cells were isolated under nonsterile conditions. However, to check the purity of our BAE cell preparation, the cells were also isolated under sterile conditions; DMEM containing antimycotic-antibiotic (1×) and 20% fetal

bovine serum was used to dislodge the cells. Aliquots (0.5 ml) of the cell suspension were then applied to sterile glass coverslips placed in 100 × 20-mm culture dishes (Corning Glass Works, Corning Medical and Scientific, Corning, NY) and cells allowed to adhere overnight at 37°C under a H<sub>2</sub>O-saturated 10% CO<sub>2</sub> atmosphere. The coverslips were then washed once with Krebs buffer, pH 7.4, quick-frozen in pentane (-70°C) and dried. The cells were then subjected to indirect immunocytofluorescence staining for Factor VIII antigen (14) and examined under a Leitz Orthoplan fluorescence microscope (E. Leitz, Inc., Rockleigh, NJ). In examining cells from three separate isolates, we found that >99% of the cells examined in random fields stained for Factor VIII. This indicates that our BAE cell isolates were essentially pure endothelial cell populations.

**Preparation of solubilized endothelial cell homogenates.** For assays of PGH synthase, BAE cells (<100 mg wet wt) isolated from single aorta were thawed and then homogenized using a glass homogenizer in 0.5 ml of 0.1 M Tris-chloride, pH 8.0 containing 2.5 mM diethyldithiocarbamate, 5 mM EDTA, and 1% Tween 20. The sample was allowed to stand at 4°C for 30 min. The solubilized BAE cell homogenate was used to assay (a) PGH synthase antigen by IRA or (b) cyclooxygenase activity using the radioisotope assay described below.

For assays of PGI<sub>2</sub> synthase, the isolated BAE cells were homogenized and solubilized in 0.5 ml of 0.1 M Tris-chloride containing 10<sup>-4</sup> M Flurbiprofen and 0.5% Triton X-100. This sample was used for both IRA and the enzyme activity assay.

**Preparation of solubilized smooth muscle homogenates.** After removal of the endothelium, the smooth muscle was cut into 2-cm<sup>3</sup> pieces and stored at -80°C. Before assay, tissue (5 g) was cut into slivers and homogenized in 5 vol of either (a) 0.1 M Tris-chloride, pH 8.0 containing 2.5 mM diethyldithiocarbamate and 5 mM EDTA (PGH synthase) or (b) 0.1 M Tris-chloride, pH 8.0 containing 10<sup>-4</sup> M Flurbiprofen (PGI<sub>2</sub> synthase) using a homogenizer (Polytron, Brinkmann Instruments, Inc., Westbury, NY). The crude homogenate was filtered through one layer of cheesecloth. The fibrous matter that did not pass through the cheesecloth was resuspended in 5 vol of homogenizing buffer, rehomogenized, and refiltered as before. The two filtrates were combined and poured through eight layers of cheesecloth. After filtration, the cheesecloth was rinsed with 2–3 vol of homogenizing buffer, and 65 ml of this filtrate was centrifuged at 125,000 g for 60 min. The pellet was homogenized using a ground glass homogenizer in 6 ml of either (a) 0.1 M Tris-chloride, pH 8.0 containing 2 mM diethyldithiocarbamate, 5 mM EDTA, and 2% Tween 20 (for assays of PGH synthase) or (b) 0.1 M Tris-chloride buffer (pH 8.0) containing 10<sup>-4</sup> M Flurbiprofen and 0.5% Triton X-100 (for assays of PGI<sub>2</sub> synthase).

**IRA for PGH and PGI<sub>2</sub> synthases.** IRA for PGH synthase antigen in solubilized homogenates of BAE cells and bovine aortic smooth muscle tissue were performed as described in detail previously (15, 16). Briefly, aliquots of solubilized tissue homogenates were added to samples containing IgG<sub>2b</sub> (cyo-5)-*Staphylococcus aureus* complexes to immobilize PGH synthase. <sup>125</sup>I-Labeled IgG<sub>1</sub> (cyo-3) was then added to the immobilized enzyme-antibody complex. Following a brief incubation the sample was centrifuged at 1,000 g for 5 min and the amount of <sup>125</sup>I label present in the resulting precipitate was measured using a Beckman Biogamma counter (Beckman Instruments, Inc., Fullerton, CA). Standard curves were generated using PGH synthase (cyclooxygenase) purified from sheep vesicular glands (17). The purified enzyme standard had a specific activity of 45,000 cyclooxygenase units per mg of protein at 37°C when assayed using a YSI model 53 oxygen monitor (17). The results of the IRA are reported in

units of cyclooxygenase activity. 1 unit is defined as the amount of activity that will catalyze formation of 1 nmol of prostaglandin/min at 37°C in an assay mixture, which consists of 3.0 ml of 0.1 M Tris-chloride, pH 8.0 containing 1 mM phenol, 100  $\mu$ M arachidonic acid, and 0.5  $\mu$ M bovine hemoglobin.

The IRA for PGI<sub>2</sub> synthase were performed as described previously (18). The general procedure is similar to the IRA for PGH synthase. Solubilized microsomes from bovine aorta were used to generate a standard curve for PGI<sub>2</sub> synthase and the results of the IRA are reported in units of PGI<sub>2</sub> synthase activity. 1 unit is that amount of enzyme that will catalyze the formation of 1 nmol of PGI<sub>2</sub>/min at 24°C in an assay mixture that consists of 0.1 ml of 0.1 M Tris-chloride, pH 8.0, containing 0.5% Triton X-100 and 50  $\mu$ M [<sup>3</sup>H]PGH<sub>2</sub> (6).

**Cyclooxygenase assays.** Aliquots (10–100  $\mu$ l) of solubilized BAE cells or bovine aortic smooth muscle tissue were added to 1 ml of 0.1 M Tris-chloride, pH 8.0 containing 10  $\mu$ M [<sup>3</sup>H]arachidonic acid ( $2 \times 10^7$  dpm/ $\mu$ mol), 0.5  $\mu$ M bovine hemoglobin, and 1 mM phenol (17, 19). Reactions were allowed to proceed for 1 min at 24°C and were stopped by the addition of 7 ml of CHCl<sub>3</sub>/methanol (1:1, vol/vol) and then extracted as described previously (19). The reaction products were separated by thin-layer chromatography on Silica Gel 60 (Merck Sharp & Dohme International Div., Rahway, NJ) in the organic phase of 2,2,4-trimethylpentane/ethyl acetate/acetic acid/H<sub>2</sub>O (50:110:20:110; vol/vol/vol/vol). Controls included samples containing no microsomes or samples to which the cyclooxygenase inhibitor Flurbiprofen ( $10^{-4}$  M) was added. The quantity of cyclooxygenase-derived product was calculated by subtracting the percentage of radioactivity chromatographing with arachidonic acid in experimental samples from that in matched control samples and multiplying by the starting quantity of arachidonate (10 nmol). Cyclooxygenase activities are reported as nanomoles arachidonate consumed per minute per milligram of protein. Assays were performed under conditions where the amount of arachidonate converted to more polar products was linearly related to the amount of solubilized protein added.

**PGI<sub>2</sub> synthase activity assays.** PGI<sub>2</sub> synthase activity was determined by measuring the conversion of [<sup>3</sup>H]PGH<sub>2</sub> to [<sup>3</sup>H]PGI<sub>2</sub> as detailed previously (6). The initial concentration of [<sup>3</sup>H]PGH<sub>2</sub> in all assays was 50  $\mu$ M.

**Protein assays.** Protein in detergent-solubilized homogenates was measured by a modification (20) of the procedure of Lowry et al. (21). 2 ml of 10% trichloroacetic acid (TCA) were added to aliquots of the cell or tissue homogenates (5–50  $\mu$ l) and the samples heated in a water bath at 70°C for 20 min. The samples were cooled to 24°C, then centrifuged at 3,000 *g* for 5 min to precipitate the protein. The standard Lowry assays were then performed. Aliquots of bovine serum albumin standard were also subjected to TCA precipitation.

**Immunocytofluorescence.** Indirect immunocytofluorescence staining of bovine aorta and bovine kidney was performed using a procedure similar to that detailed earlier (12, 22). Briefly, blocks of fresh tissue were quick-frozen in pentane (–70°C) and sectioned on a cryotome at –25°C. The tissue sections were transferred to coverslips, dried for 30 min, and then overlaid with a first antibody preparation; these included culture media from the growth of mouse hybridoma lines *cyo-1*, *cyo-3*, or *cyo-5* (all anti-PGH synthase [15]), *tsn-1* or *tsn-3* (both anti-PGI<sub>2</sub> synthase [6]) or *day-1* (nonimmune control). After a 30-min incubation with first antibody, the sections were washed with phosphate-buffered saline, pH 7.2, and overlaid with a 1:20 dilution of fluorescein isothiocyanate-labeled rabbit anti-mouse IgG and

incubated an additional 30 min. Samples were then washed, mounted on glass slides in glycerol and examined using a Leitz Orthoplan fluorescence microscope. Fluorescence microscopy was performed using an Orthomat camera and Kodak TX-135 film (Eastman Kodak Co., Rochester, NY). The photomicrographs shown in Fig. 1 were taken with identical exposure times (1 min) and the prints were developed under identical conditions so that semiquantitative comparisons of relative staining intensities can be made.

## RESULTS

The levels of PGH synthase and PGI<sub>2</sub> synthase in freshly isolated endothelial cells and smooth muscle tissue from bovine aorta were first determined using IRA for these proteins (16, 18). The results of studies performed using tissue samples from 11 different animals are presented in Table I. With each aorta the level of PGH synthase was considerably greater (~20 times) in endothelial cells than in smooth muscle. In contrast, the concentrations of PGI<sub>2</sub> synthase were not significantly different between endothelial cells and smooth muscle. Two other pertinent observations are that there is more PGI<sub>2</sub> synthase activity than PGH synthase activity in smooth muscle but higher PGH synthase activity than PGI<sub>2</sub> synthase activity in endothelial cells. The values in Table I are expressed in terms of catalytic units per milligram of tissue protein. The specific activity of purified PGH synthase (~45,000 units/mg protein [17]) is higher than that of PGI<sub>2</sub> synthase (~1,000 units/mg protein [6]). Thus, 1 unit of PGI<sub>2</sub> synthase activity represents a larger number of enzyme protein molecules than a unit of PGH synthase.

The results of IRA for PGH synthase (or for PGI<sub>2</sub> synthase) in different cell types can only be compared if the proteins in these cells are immunochemically equivalent (i.e., if the amount of catalytic activity per unit mass of immunoprecipitable protein is the same for both tissues). Because only a limited quantity of fresh endothelial cells could be obtained from a single aorta, it was not practical to generate standard immunochemical equivalence curves (e.g., 23) for these cells. As an alternative, we measured PGI<sub>2</sub> synthase activity (Table II) and PGH synthase (i.e., cyclooxygenase) activities (Table III) in detergent-solubilized homogenates of smooth muscle and endothelium. The values obtained for PGI<sub>2</sub> synthase (Table II) from six bovine aorta fall within the range expected on the basis of the IRA for this enzyme; the results confirm that the concentrations of PGI<sub>2</sub> synthase are the same in smooth muscle and endothelial cells. The specific cyclooxygenase activity (Table III) was found to be 20–30 times higher in endothelial cells than in smooth muscle, a result consistent with that obtained by IRA.

The existence of a gradient in PGI<sub>2</sub> synthase concentrations in arterial smooth muscle between the internal elastic lamina and the adventitia was suggested

TABLE I  
PGH Synthase and PGI<sub>2</sub> Synthase Concentrations in Smooth Muscle and Endothelial Cells  
from Bovine Aorta As Determined by IRA\*

Bovine aorta	PGH synthase (cyclooxygenase)			PGI <sub>2</sub> synthase		
	Endothelium	Smooth muscle	Endothelium/smooth muscle ratio	Endothelium	Smooth muscle	Endothelium/smooth muscle ratio
	units/mg cell protein			units/mg cell protein		
1	21	2.4	8.8	7.6	7.5	1.0
2	20	2.0	10	9.3	9.2	1.0
3	26	1.4	19	2.6	5.2	0.50
4	20	0.92	22	1.8	5.7	0.32
5	23	0.84	27	2.1	6.4	0.33
6	15	0.75	20	1.7	3.2	0.53
7	21	1.4	15	1.3	5.3	0.25
8	45	1.2	38	3.4	5.0	0.68
9	32	1.1	29	3.2	4.7	0.68
10	60	1.7	35	2.8	3.8	0.74
11	ND	ND	—	7.9	8.4	0.94
	28±14	1.4±0.53	20	4.4±2.8	6.4±2.0	0.69

\* IRA for PGH synthase and PGI<sub>2</sub> synthase were performed as described in the text. 1 unit of PGH synthase represents the amount of enzyme that will catalyze the formation of 1 nmol of PGH<sub>2</sub> (from arachidonic acid) per min at 37°C under the standard assay conditions. 1 unit of PGI<sub>2</sub> synthase represents the amount of enzyme that will catalyze the formation of 1 nmol of PGI<sub>2</sub> (from PGH<sub>2</sub>)/min at 24°C under the standard assay conditions. Values are means±SD. Values for PGH synthase in smooth muscle and endothelium are significantly different from one another ( $P < 0.001$ ) as determined by Student's *t* test. Values for PGI<sub>2</sub> synthase in smooth muscle and endothelium are not different. ND, not determined.

by the early studies of Moncada et al. (7) on rabbit aorta. To examine this question, we stained cross-sections of arteries (both abdominal aorta and renal arteries) for PGH synthase and PGI<sub>2</sub> synthase immunoreactivities using an indirect immunocytofluorescence procedure (12, 22). As shown in Fig. 1 A for the case of bovine renal arteries, the intensity of fluorescent staining for PGI<sub>2</sub> synthase was the same throughout the smooth muscle layer; moreover, as expected from our quantitative IRA, the intensities of PGI<sub>2</sub>-positive fluorescence were similar in smooth muscle and endothelium. In contrast, PGH synthase immunofluorescence was considerably more intense in the endothelium than in the smooth muscle (Fig. 1 B). Results similar to those noted in renal arteries were observed in examining sections of abdominal aorta by immunocytofluorescence. It should also be noted that the patterns of fluorescent staining shown for PGI<sub>2</sub> synthase and PGH synthase in bovine vasculature were observed in the arterial vasculatures of rabbit, guinea pig, rat, and sheep. These latter observations suggest that the quantitative data obtained for bovine aorta (Tables I-III) are representative of the distribution of PGI<sub>2</sub> synthase and PGH synthase in all mammalian species.

TABLE II  
PGI<sub>2</sub> Synthase Concentrations in Smooth Muscle and Endothelial Cells from Bovine Aorta as Determined by Assays of Catalytic Activity\*

Bovine aorta	PGI <sub>2</sub> synthase		
	Endothelium	Smooth muscle	Endothelium/smooth muscle ratio
	units/mg cell protein		
12	2.1	2.8	0.75
13	3.4	2.7	1.3
14	1.5	1.8	0.83
15	2.9	2.1	1.4
16	2.6	2.6	1.0
17	4.4	3.4	1.3
	2.8±1.0	2.6±0.56	1.1

\* Solubilized homogenates of smooth muscle and endothelial cells were prepared and assayed for PGI<sub>2</sub> synthase activity using [<sup>3</sup>H]PGH<sub>2</sub> (50 μM) as substrate as detailed in Methods. 1 unit represents the amount of enzyme that will catalyze the formation of 1 nmol of PGI<sub>2</sub>/min at 24°C under the standard assay conditions. Data represent means±SD. Values for smooth muscle and endothelium are not significantly different from one another as determined by Student's *t* test.

TABLE III  
PGH Synthase (Cyclooxygenase) Concentrations in  
Smooth Muscle and Endothelial Cells from  
Bovine Aorta as Determined by Assays  
of Catalytic Activity

Bovine aorta	PGH synthase (cyclooxygenase)		
	Endothelium	Smooth muscle	Endothelium/smooth muscle ratio
	units/mg cell protein		
18	33	1.1	30
19	30	1.1	27
20	42	1.9	22
	35±6.2	1.4±0.66	25

\* Solubilized homogenates of smooth muscle and endothelial cells were prepared and assayed for cyclooxygenase activity using [<sup>3</sup>H]arachidonic acid (10  $\mu$ M) as substrate as detailed in Methods. 1 unit represents the amount of enzyme that will catalyze the formation of 1 nmol of prostaglandin product/min at 24°C under the standard assay conditions.

Data represent means±SD. Values for smooth muscle and endothelium are significantly different from one another ( $P < 0.01$ ) as determined by Student's *t* test.

## DISCUSSION

Platelets do not adhere to the endothelium lining healthy arteries but do adhere to the subendothelium following vascular injury (24–26). Although the binding of platelets to normal endothelial cells is not influenced by prostacyclin (24), PGI<sub>2</sub> may be important physiologically as an inhibitor of platelet-platelet interactions and, perhaps, as a modulator of the interactions of platelets with the subendothelium. Exogenous PGI<sub>2</sub> can partially inhibit the binding of platelets to newly exposed areas of the subendothelium (1, 3, 27, 28). There is, however, conflicting data concerning whether inhibition of vascular PGI<sub>2</sub> production by nonsteroidal antiinflammatory agents enhances platelet adherence to newly deendothelialized areas of arterial walls (29–32). Nevertheless, after a damaged artery begins to repair itself, there is an increase in the capacity of the deendothelialized vessel to form PGI<sub>2</sub> from arachidonic acid that correlates with the formation of a nonthrombogenic neointimal surface (26). Thus, at least in the case of arteries undergoing repair, PGI<sub>2</sub> may influence the propagation of platelet aggregates and perhaps to a lesser extent the initial adhesion of platelets to subintimal structures.

The propensity of platelets to adhere to the subendothelium of newly damaged vessels has been attributed to the relative inability of this cell layer to synthesize PGI<sub>2</sub> (2, 33). Bunting et al. (1) and Moncada et al. (7) reported that microsomes prepared from whole aorta exhibit low PGH synthase (cyclooxygenase) activity and that the media of arteries has only

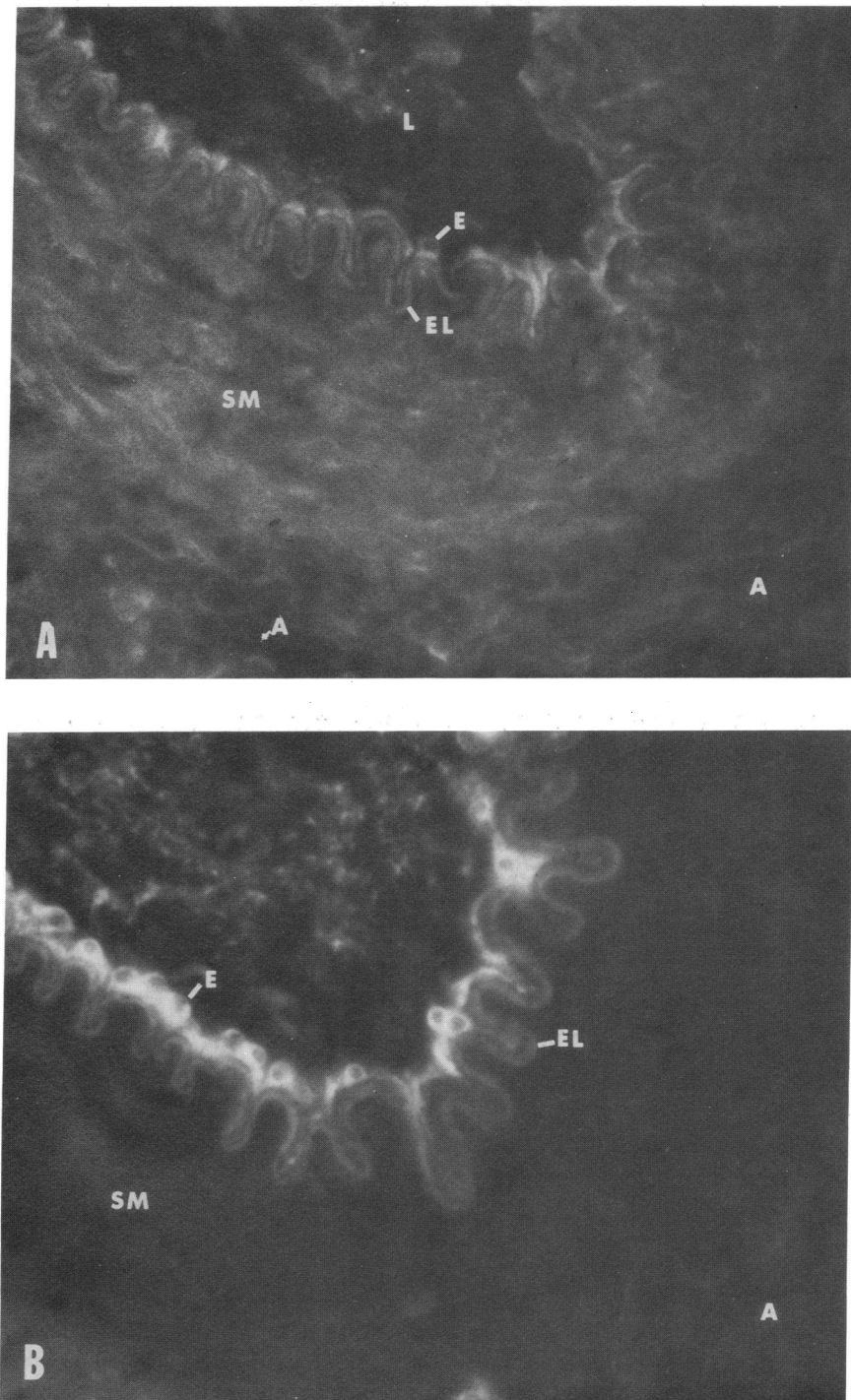
10–15% of the capacity of the endothelium to synthesize PGI<sub>2</sub> from the prostaglandin endoperoxide PGH<sub>2</sub>. Our results, which have used measurements of both enzyme protein and enzyme activity, indicate that smooth muscle cells, in fact, have the same specific PGI<sub>2</sub> synthase activity as endothelial cells. Thus, adherence of platelets to the subendothelium cannot be ascribed simply to an inability of smooth muscle to form PGI<sub>2</sub> from PGH<sub>2</sub> that may be released from platelets (33). It also seems unlikely that there are differences in the processing of PGH<sub>2</sub> (e.g., uptake) by smooth muscle and endothelial cells since cultures of smooth muscle and endothelial cells synthesize PGI<sub>2</sub> from PGH<sub>2</sub> at comparable rates (8–11).

Although there appears to be a uniform concentration of PGI<sub>2</sub> synthase in the vasculature, the levels of PGH synthase in smooth muscle are only ~5% of those in endothelial cells. The major quantitative difference between PGH synthase concentrations in endothelial and smooth muscle cells is consistent with the observation that newly deendothelialized aorta has <10% of the capacity of normal aorta to form PGI<sub>2</sub> from arachidonic acid (26).

The functional significance of the difference in PGH synthase concentrations between smooth muscle and endothelium is not obvious. We propose the following speculative scenario. In general, the amount of prostaglandin end-product released in response to a cellular stimulus is determined by the efficiency with which arachidonate liberated from precursor lipids is converted to PGH<sub>2</sub> (23, 34). Therefore, for two different cells containing the same level of PGI<sub>2</sub> synthase, the concentration of PGH synthase is likely to be an indicator of the relative ability of each cell to synthesize and release PGI<sub>2</sub>. Both the endothelium of healthy arteries and the neointima of damaged arteries (26) have relatively high levels of PGH synthase activity. We speculate that as a consequence the lining of arteries usually maintains a high capacity to release PGI<sub>2</sub> into the vascular lumen, where PGI<sub>2</sub> presumably can influence platelet function. Conversely, smooth muscle, which has relatively low PGH synthase activity, probably makes less PGI<sub>2</sub> in response to cellular stimuli and the PGI<sub>2</sub> synthesized may function principally, if not exclusively, within the smooth muscle layer to regulate vascular tone. Consistent with this narrow role proposed for PGI<sub>2</sub> originating in vascular smooth muscle are the findings (a) that all smooth muscle, both vascular and nonvascular, forms PGI<sub>2</sub> and (b) that the ratios of PGI<sub>2</sub> synthase to PGH synthase appear to be similarly high in all smooth muscle (12).

## ACKNOWLEDGMENTS

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**FIGURE 1** Distribution of  $\text{PGI}_2$  synthase and  $\text{PGI}_2$  synthase antigens in arterial vasculature. Cryotome sections of bovine renal arteries were subjected to indirect immunocytofluorescence staining for (A)  $\text{PGI}_2$  synthase or (B)  $\text{PGH}$  synthase using monoclonal antibodies to each protein. Details are presented in the text. L, lumen; EC, endothelial cell; SM, smooth muscle; A, adventitia; EL, internal elastic lamina. Magnification,  $\times 200$ .



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