Immunoochemical and Kinetic Evidence for Two Different Prostaglandin H-Prostaglandin E Isomerases in Sheep Vesicular Gland Microsomes*

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Splenic lymphocytes from mice immunized with a partially purified prostaglandin (PG) H-PGE isomerase from sheep vesicular glands were fused with SP2/0-Ag14 myeloma cells. Two spleen cell-myeloma hybrids (hei-7 and hei-26) were selected and cloned. The mouse antibodies secreted by the two hybrids, IgG1(hei-7) and IgG1(hei-26), caused immunoprecipitation of a maximum of 45 and 22%, respectively, of the solubilized PGH-PGE isomerase activity of sheep vesicular gland; immunoprecipitation of activity by the two antibodies was additive. The antigens reactive with IgG1(hei-7) and IgG1(hei-26) were identified as proteins with $M_\text{r} = 17,500$ and 180,000, respectively, by Western transfer blotting or sodium dodecyl sulfate-polyacrylamide gel electrophoresis of immunoprecipitated $^{125}_{1}$-labeled microsomes. The PGH-PGE isomerase activities precipitated by IgG1(hei-7) and IgG1(hei-26) exhibited different kinetic properties with respect to time course, $K_{\text{m}}$ for PGH$_2$, and concentration dependence for GSH. No significant GSH-S-transferase activity was present in these immunoprecipitates. These data indicate that there are at least two different proteins in sheep vesicular gland microsomes capable of catalyzing GSH-dependent PGH-PGE isomerase reactions.

IgG1(hei-7), but not IgG1(hei-26), caused coprecipitation of PGH synthase and PGH-PGE isomerase activities when incubated with intact right-side-out vesicular gland microsomes. Thus, the epitope for IgG1(hei-7) is located on the cytoplasmic surface of those microsomal spheres which contain PGH synthase. This latter finding suggests that the isomerase reactive with IgG1(hei-7) is involved in PGE synthesis in sheep vesicular glands.

PGE$_1$, was first described in 1961 as a major prostaglandin metabolite of sheep vesicular gland (1). PGE$_1$ is derived biosynthetically from 8-cis,11-cis,14-cis-eicosatrienoic acid. Quantitatively, however, the most important prostaglandin of the E series is PGE$_2$, which is formed from arachidonic acid. PGE$_2$ is the major prostaglandin released by a number of cell types, including endothelial cells derived from the microvasculature (2), interstitial cells of the renal medulla (3), and epithelial cells of the collecting tubule (4-6). PGE$_2$ is a vasodilator in most vascular beds (7, 8), inhibits Na$^+$ resorption in the renal collecting tubule (9) and Cl$^-$ resorption in the medullary thick limb of Henle's loop (10), and inhibits vasopressin-induced cAMP formation in the cortical collecting tubule (11, 12) and the medullary thick limb (11). In many cells which synthesize prostaglandins, PGE$_2$ also causes the activation of adenylate cyclase (13, 14).

The biosynthesis of PGE$_2$ from arachidonic acid (or PGE$_1$ from 8,11,14-eicosatrienoic acid) involves two successive reactions which are catalyzed by PGH synthase and PGH-PGE isomerase, respectively. There is now a considerable body of information on the kinetic and physical characteristics of PGH synthase and biochemical and pharmacological regulation of this enzyme (see Ref. 15 for review). In contrast, little is known about the properties of PGH-PGE isomerase. PGH-PGE isomerase activities from vesicular gland have been solubilized and partially purified by Ogino et al. (16) and by Moonen et al. (17). The enzyme has been shown to require GSH for activity and to be inactivated by treatment with sulfhydryl reagents. GSH is not oxidized stoichiometrically during enzyme-catalyzed formation of PGE$_2$ from PGH$_2$, and a mechanism consistent with this observation and analogous to the role of GSH in the glyoxylase I reaction has been proposed (18). According to this mechanism GSH interacts with C-9 of PGH$_2$ to facilitate a 1,2-hydride shift resulting in the 9-keto,11-hydroxy structure characteristic of PGE derivatives.

As part of our studies on the regulation of PGE$_2$ metabolism by renal collecting tubules (4, 6), we prepared monoclonal antibodies against what was expected to be a single PGH-PGE isomerase. We used sheep vesicular glands as the source of the enzyme since extracts of this gland contain high levels of GSH-dependent PGH-PGE isomerase activity (16, 17). Surprisingly, we obtained two different monoclonal antibodies, each of which causes significant, but incomplete, precipitation of GSH-dependent PGH-PGE isomerase activity. This report documents our studies on these activities, as well as related work, to determine if the proteins reactive with the monoclonal antibodies are localized in the same cells and on the same membrane systems as PGH synthase, the enzyme which catalyzes formation of the prostaglandin endoperoxide substrate for PGH-PGE isomerase.
**EXPERIMENTAL PROCEDURES AND RESULTS**

The methods used in the studies reported in this paper are detailed in the Miniprint. Also presented in the Miniprint is a description of the immunocytochemical staining patterns obtained with monoclonal antibodies to PGH-PGE isomerases.

Solubilization of PGH-PGE Isomerase Activity—The specific PGH synthase (cyclooxygenase) activity of sheep vesicular gland microsomes was determined to be 5400 nmol of PGE_2 formed per min per mg of protein at 37 °C. The specific PGH isomerase activity of microsomes was obtained with monoclonal antibodies to PGH-PGE isomerases.

As observed previously (16, 17), PGH-PGE isomerase activity present in the postnuclear supernatant fraction of sheep vesicular glands homogenates was found almost exclusively (90%) in the 6 x 10^6 g x min microsomal pellet. The microsomal enzyme activity could be partially solubilized in 50% buffer solution and then suspended with the same buffer at 4 °C. The resulting pellets were washed with 1 ml of the same buffer solution and then suspended with the same buffer (55 µl). PGH-PGE isomerase activity was assayed as described under "Experimental Procedures." The ratio of IgG_1(hei-7) to IgG_1(hei-26) in D was 1:3. PGE_2 formation without enzyme (0.26 mmol/15 s) was subtracted from the observed rates to give the values presented in the figure.

Monoclonal Antibodies to PGH-PGE Isomerase Activities—Mice were immunized with samples of a partially purified PGH-PGE isomerase prepared from sheep vesicular gland microsomes essentially as described by Moonen et al. (17). Splenic lymphocytes from the immunized mice were fused with cells from the SP2/0-Ag14 myeloma line (19, 20). Three mouse spleen cell-plasmacytoma hybrids were found to secrete mouse immunoglobulins (IgG1) which do not interact with any sheep vesicular gland protein as determined (a) by analysis of immunoprecipitates of solubilized sheep vesicular gland microsomes by SDS-polyacrylamide gel electrophoresis and (b) by indirect immunocytofluorescent staining of cryotome sections of sheep vesicular gland.

2 Portions of this paper (including "Experimental Procedures," part of "Results," part of "Discussion," and Table IS) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9550 Rockville Pike, Bethesda, MD 20814. Request Document No. 86M-1887, cite the authors, and include a check or money order for $4.00 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

3 The hybridomas designated day-1 and tsn-1 are lines which secrete mouse immunoglobulins (IgG1) which do not interact with any sheep vesicular gland protein as determined (a) by analysis of immunoprecipitates of solubilized sheep vesicular gland microsomes by SDS-polyacrylamide gel electrophoresis and (b) by indirect immunocytofluorescent staining of cryotome sections of sheep vesicular gland.

**FIG. 1.** Immunoprecipitation of solubilized PGH-PGE isomerase activity by monoclonal antibodies in the presence of rabbit anti-mouse IgG. Mouse immunoglobulins were purified from culture media as described in the Miniprint. Aliquots of solubilized sheep vesicular gland microsomes (5 µl; 1.7 mg of protein/ml; PGH-PGE isomerase activity = 1.7 nmol/15 s/µl) were mixed with the indicated amounts of purified (A) IgG_1(hei-2), (B) IgG_1(hei-7), (C) IgG_1(hei-26), or (D) IgG_1(hei-7) and IgG_1(hei-26) in 0.1 M Tris-HCl, pH 8.0, containing 2 mM GSH and 0.5% Triton X-100, and the samples were incubated on ice for 5 min. Rabbit anti-mouse IgG serum (40 µl; 92 µg of protein) was then added to each sample. After standing for 2 min on ice, the samples were centrifuged for 10 min at 1500 x g at 4 °C. The resulting pellets were washed with 1 ml of the same buffer solution and then suspended with the same buffer (55 µl). PGH-PGE isomerase activity was assayed as described under "Experimental Procedures." The ratio of IgG_1(hei-7) to IgG_1(hei-26) in D was 1:3. PGE_2 formation without enzyme (0.26 mmol/15 s) was subtracted from the observed rates to give the values presented in the figure.
precipitation of a unique protein (Mr = 72,500). A faint band (M, = 47,200) corresponding to an immunoglobulin heavy chain was found in all the immunoprecipitates. Between eight and eleven 125I-labeled proteins were present in immunoprecipitates of IgG1(hei-2) (data not shown), and no further analyses were performed with this antibody. Immunoprecipitation of radioactivity using IgG1(hei-26) resulted in the precipitation of a single band (M, = 120,000), but in most instances no radioactive proteins were precipitated. However, when we analyzed IgG1(hei-26) by Western transfer blotting of solubilized microsomes, a single band (M, = 180,000) was routinely detected (Fig. 4); no protein-staining bands were seen in Western transfer blotting with IgG1(hei-2), IgG1(hei-7), or IgG1(cyo-3). Together, these experiments indicate that IgG1(hei-7) and IgG1(hei-26) are directed against unique antigens having subunit molecular weights of 17,500 and 180,000, respectively. Further evidence supporting this concept was obtained in a second immunoblotting experiment. Solubilized microsomes (1.1 mg, 4.5 mg of protein/ml) were incubated with an excess (500-700 pg) of IgG1(hei-7) and IgG1(cyo-3). The complexes were then precipitated by the addition of 550 pg of rabbit anti-mouse IgG. The precipitates were subjected to SDS-polyacrylamide gel electrophoresis on a 15% SDS-polyacrylamide gel which was then dried and exposed to x-ray film to obtain the autoradiogram. Details of the methodology are presented in the text.

Kinetic Properties of PGH-PGE Isomerases—Some simple kinetic analyses of the PGH-PGE isomerases precipitated by the different anti-PGH-PGE isomerase antibodies were performed to determine if the activities exhibited different characteristics. The results of experiments to determine pH optima, K_M values, and other parameters are summarized in Table I. The K_M for PGH_2 of the activity precipitated by IgG1(hei-26) was 150 \mu M, 3-4 times the K_M values observed with the solubilized microsomal enzyme or with the PGH-PGE isomerase activity precipitated by IgG1(hei-7). In addition, the enzyme precipitated by IgG1(hei-26) required a relatively lower concentration of GSH (\leq0.5 \ mM) for maximal activity than the enzyme precipitated by IgG1(hei-7) (2 \ mM). The time course of the reaction catalyzed by the PGH-PGE
isomerase precipitated by IgG₁(hei-7) was also significantly different from the time course observed with the activity precipitated by IgG₁(hei-26) (Fig. 5). Maximal PGE₂ formation had occurred by 15 s with the enzyme precipitated by IgG₁(hei-7), while PGE₂ formation had not yet plateaued at 60 s in the reaction catalyzed by the enzyme precipitated by IgG₁(hei-26). The time course for the enzyme precipitated by IgG₁(hei-7) resembles the time course seen with PG₁₂ synthase where the enzyme activity is rapidly lost during the course of the reaction (21).

The PGH-PGE isomerases precipitated by the different antibodies resembled one another (a) in being refractory to inhibition by Ca²⁺ or Mg²⁺ at concentrations up to 10 mM (Table I) and (b) in being activated by phosphatidylcholine (maximal activation occurring with 3 mM phosphatidylcholine at 0.01% Triton X-100; data not shown). None of the immunoprecipitates exhibited significant GSH-S-transferase activity (Table I). The specific GSH-S-transferase activities found in the immunoprecipitates formed with IgG₁(hei-7) and IgG₁(hei-26) represented 0.7 and <0.1%, respectively, of the starting activity (as compared with 45 and 22%, respectively, of the starting PGH-PGE isomerase activity).

**Topological Relationships between PGH Synthase and PGH-PGE Isomerases—PGH-PGE isomerases important in PGE synthesis in vivo would be expected to be located in the same cell and on the same membrane system as the PGH synthase. Therefore, we determined the cellular and subcellular location of the antigens reactive with the anti-PGH-PGE isomerase antibodies. As discussed in the Miniprint, immunocytochemical studies indicated that in sheep vesicular glands, the PGH synthase reactive with IgG₁(cyo-3) and the PGH-PGE isomerases active with both IgG₁(hei-7) and IgG₁(hei-26) are all present in greatest relative abundance in glandular epithelial cells.**

We next sought to determine if PGH synthase and the PGH-PGE isomerases were all associated with the same membrane system. Anti-PGH synthase, anti-PGH-PGE isomerase, anti-PG₁₂ synthase (IgG₁(isn-3) (21)), and control antibodies were complexed to *S. aureus* conjugates and the different antibody-*S. aureus* conjugates were tested for their abilities to coprecipitate PGH synthase and PGH-PGE isomerase activities from microsome preparations (Table II). IgG₁(cyo-3) and IgG₁(hei-7)-*S. aureus* complexes when incubated with...
Rabbit anti-mouse IgG was conjugated with S. aureus cells as described in the Miniprint. Aliquots (0.4 ml) of rabbit anti-mouse IgG-S. aureus conjugate (10% w/v) were mixed with 0.4-ml aliquots of hybridoma medium containing the indicated monoclonal antibodies (SP2 denotes medium from growth of mouse myeloma SP2/0-Agt14 line). After incubation for 60 min at 24°C, the cell pellets were collected by centrifugation. In experiments designed to measure immunoprecipitation of PGH-PGE isomerase activity, the mouse IgG-S. aureus complexes were resuspended in 60 μl of PGH-PGE isomerase assay buffer (without Triton X-100) and incubated for 3 min at 4°C with 80 μl (180 μg of protein) of a suspension of sheep vesicular gland microsomes. The pellets were collected by centrifugation and washed with 1 ml of assay buffer lacking detergent and resuspended in assay buffer. The resuspended pellet and the supernatant were assayed for PGH-PGE isomerase activity. The original PGH-PGE isomerase activity of intact microsomes was 290 nmol/mg/15 s. In experiments designed to measure immunoprecipitation of PGH synthase activity, the mouse IgG-S. aureus complexes were suspended in 60 μl of PGH synthase assay buffer (20) and incubated for 3 min at 4°C with 40 μl (90 μg of protein) of a suspension of sheep vesicular gland microsomes. Pellets and supernatants were collected as indicated above and assayed for PGH synthase activity. The original PGH synthase activity of intact microsomes was 5.8 μmol/mg/min.

Table II

| Antibody-S. aureus complex used for immunoprecipitation | PGH-PGE isomerase activity | PGH synthase activity |
|----------------------------------------------------------|---------------------------|-----------------------|
|                                                          | Soluble fraction | % original activity | Soluble fraction | % original activity |
| IgG1(hei-7)                                              | 3.1             | 85                   | 0               | 51                   |
| IgG1(hei-26)                                             | 44              | 14                   | 41              | 3.2                  |
| IgG1(isn-3)                                              | 20              | 88                   | 0               | 67                   |
| IgG1(cyo-3)                                              | 38              | 12                   | 46              | 2.4                  |
| IgG1(day-1)                                              | 44              | 13                   | 36              | 7.8                  |
| SP2                                                      | 42              | 12                   | 47              | 5.5                  |

Intact vesicular gland microsomes did cause coprecipitation of PGH-PGE isomerase and PGH synthase activities; in contrast, IgG1(hei-26), IgG1(isn-3), and IgG1(day-1)-S. aureus complexes precipitated only background levels of both activities. The anti-PGH synthase-S. aureus complex failed to precipitate PGH-PGE isomerase activity from solubilized membrane preparations but, of course, did precipitate solubilized PGH synthase activity (data not shown); similarly, anti-PGH-PGE isomerase-S. aureus complexes caused partial precipitation of detergent-solubilized PGH-PGE isomerase activity, but did not precipitate solubilized PGH synthase activity. These immunoprecipitation experiments were conducted under conditions developed previously to establish that PGH synthase is located on the cytoplasmic surface of intact right-side-out sheep vesicular gland microsomes (20). Thus, the present results indicate that antigenic determinants of both PGH synthase and the protein reactive with IgG1(hei-7) are associated with the same membrane systems and are on the same side of the membrane.4

Discussion

The major prostaglandin products formed upon incubations of 8,11,14-eicosatrienoic acid or arachidonic acid with sheep vesicular gland microsomes are PGE and PGE₂, respectively (23). Small amounts of PGI₂ are also synthesized (24), probably by membranes derived from smooth muscle and endothelial cells present in these glands (25). Vesicular glands have been shown by Ogino et al. (16) and Moonen et al. (17) to have a heat-labile activity capable of catalyzing a GSH-dependent isomerization of prostaglandin endoperoxides to PGE derivatives; about 90% of this activity is membrane associated. Using Triton X-100, we were able to solubilize approximately 50% of the microsomal PGH-PGE isomerase activity and were able to account for 70-80% of this solubilized activity in immunoprecipitates obtained using IgG1(hei-2), IgG1(hei-7), and IgG1(hei-26).

Our data suggest that there are at least three different proteins in sheep vesicular gland microsomes which can catalyze GSH-dependent PGH-PGE isomerase reactions at significant rates. Two of these isomerases are precipitated by IgG1(hei-7) and IgG1(hei-26), respectively. In addition, since 20-30% of the total solubilized activity could not be precipitated by the antibodies we have developed, there is likely at least one other isomerase activity. IgG1(hei-7) and IgG1(hei-26) precipitate different amounts of the total PGH-PGE isomerase activity and precipitation is additive. The protein antigens precipitated by IgG1(hei-7) and IgG1(hei-26) are different, having subunit molecular weights of 17,500 and 180,000, respectively. Moreover, the PGH-PGE isomerase activities precipitated by IgG1(hei-7) and IgG1(hei-26) differ substantially in their kinetic profiles.

The PGH-PGE isomerase reactive with IgG1(hei-7) has several properties which would be expected for a biologically important PGH-PGE isomerase. Immunofluorescent staining indicated that the protein reactive with IgG1(hei-7) is present in greatest abundance in glandular epithelial cells of vesicular gland, the same cells which stain most intensely for PGH synthase. Moreover, IgG1(hei-7) and IgG1(cyo-3), when conjugated to S. aureus cells, cause the coprecipitation of both PGH synthase and PGH-PGE isomerase activities from intact right-side-out microsomes. The cyclooxygenase active site and the IgG1(cyo-3)-reactive epitope of PGH synthase are on the cytoplasmic surface of the endoplasmic reticulum (20). The present findings indicate that epitope of the antigen reactive with IgG1(hei-7) is also present on the cytoplasmic surface of the endoplasmic reticulum. Thus, the PGH-PGE
isomerase precipitated by IgG<sub>1</sub>(hei-7) is present in the same membrane system as PGH synthase, the site of generation of prostaglandin endoperoxide and TxA<sub>2</sub> formation has been observed in studies on PGH synthase (25) and TxA<sub>2</sub> synthase (26).

While IgG<sub>1</sub>(hei-7) is directed against a protein that likely catalyzes PGE<sub>2</sub> formation in vesicular gland, it is not possible to make the same case for the protein reactive with IgG<sub>1</sub>(hei-26). This latter antigen is also located in glandular epithelia of vesicular gland. However, IgG<sub>1</sub>(hei-26) precipitates PGH-PGE isomerase activity only from solubilized microsomes. Thus, it is unclear whether or not the IgG<sub>1</sub>(hei-26)-reactive PGH-PGE isomerase is present in the vicinity of PGH<sub>2</sub> synthesis. Moreover, the <i>K<sub>m</sub></i> for PGH<sub>2</sub> of the PGH-PGE isomerase precipitated by IgG<sub>1</sub>(hei-26) is 150 µM, which is 5-10 times the <i>K<sub>m</sub></i> values for other PGH<sub>2</sub>-metabolizing enzymes (15, 27-29). It is possible that the PGH-PGE isomerase activity of the protein precipitated by IgG<sub>1</sub>(hei-26) is actually a spurious activity associated with a protein having another primary function. A precedent for this has been noted with serum albumin which will catalyze the synthesis of PGD<sub>2</sub> at an appreciable rate (30, 31).

A perplexing result of our studies is that the cellular distribution of the IgG<sub>1</sub>(hei-7)-reactive epitope, as determined by immunofluorescent staining (Miniprint), is inconsistent with the known distribution of sites of PGE<sub>2</sub> synthesis in tissues other than vesicular glands. The basis for these apparent discrepancies needs to be explored. At present, the most likely possibilities are that IgG<sub>1</sub>(hei-7) interacts with related epitopes of other proteins lacking PGH-PGE isomerase activity and/or that there may be tissue-specific PGH-PGE isomerase isoforms.

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SUPPLEMENTAL MATERIAL

IMMUNOCHEMICAL AND KINETIC EVIDENCE FOR THE DIFFERENT PGE ISOENZYMES IN DAILY VEGETABLE MICROBES

By Takashi Takeda, Sherry L. Ward, and William L. Smith

Described in this supplement are the methods for the preparation and analysis of nonspecific PGE isoenzymes reported to PGE-immunosuppressive studies using monoclonal antibodies to PGE-isoenzymes.

EXPERIMENTAL PROCEDURES

Materials

Kynurenic acid and 8-azaguanine were purchased from Sigma Chemical Co., Inc. (5,6,7,8-tetrahydro-5-oxo-2-phenyl-3,4-dihydro-1,2-oxadiazole) and (R)-alpha-ethyl-phenylglycine (R)-alphaethyl-phenylglycine ethyl ester, respectively. 2-Aminoethanol (2-acetamidopropanol) and Bis-Tris propane (3-[N-morpholino]-propanesulfonic acid) buffer were purchased from BDH Chemical Co., Ltd. 2-Mercaptobenzothiazole was purchased from Wako Pure Chemical Co., Ltd. 5-Fluorouracil was purchased from Calbiochem, and 5-fluoro-2'-deoxyuridine was purchased from Sigma Chemical Co., Inc. for the preparation of 5-fluorodeoxyuridine 1-β-D-arabinofuranoside (FUDR) and 2′-fluoro-2′-deoxyuridine, respectively. All other reagents were purchased from commercial sources.

Preparation of 5-fluoro-2'-deoxyuridine 1-β-D-arabinofuranoside (FUDR)

3,5-Dibromouridine (DBU) was synthesized from 5-fluorouracil (5FU) by treatment with DBU and 3,5-dibromopyrazine. 5-Fluorodeoxyuridine 1-β-D-arabinofuranoside (FUDR) was prepared by treatment of 5FU with 5-fluorodeoxyuridine 1-β-D-arabinofuranoside at 85 °C for 16 h. The reaction mixture was cooled to room temperature before being concentrated in vacuo. 5-fluoro-2'-deoxyuridine 1-β-D-arabinofuranoside (FUDR) was further purified by column chromatography on Sephadex LH-20 with methanol as eluent and was stored at −20 °C after being freeze-dried.

Preparation of 5-fluorodeoxyuridine 1-β-D-arabinofuranoside (FUDR)

5-fluorodeoxyuridine 1-β-D-arabinofuranoside (FUDR) was prepared by treatment of 5FU with 5-fluorodeoxyuridine 1-β-D-arabinofuranoside at 85 °C for 16 h. The reaction mixture was cooled to room temperature before being concentrated in vacuo. 5-fluoro-2'-deoxyuridine 1-β-D-arabinofuranoside (FUDR) was further purified by column chromatography on Sephadex LH-20 with methanol as eluent and was stored at −20 °C after being freeze-dried.

Preparation of 5-fluorodeoxyuridine 1-β-D-arabinofuranoside (FUDR)

5-fluorodeoxyuridine 1-β-D-arabinofuranoside (FUDR) was prepared by treatment of 5FU with 5-fluorodeoxyuridine 1-β-D-arabinofuranoside at 85 °C for 16 h. The reaction mixture was cooled to room temperature before being concentrated in vacuo. 5-fluoro-2'-deoxyuridine 1-β-D-arabinofuranoside (FUDR) was further purified by column chromatography on Sephadex LH-20 with methanol as eluent and was stored at −20 °C after being freeze-dried.
staining of vascular and nonvascular smooth muscle (Table 10). IgG(162-24) caused faint staining of epithelial cells only.

To determine if proteins reactive with IgG(162-24) and IgG(162-25) were present in tissues other than those vascular tissues, these antibodies were used to stain normal rat kidney, rabbit kidney, mouse kidney, and human liver and heart. The distribution of staining by these antibodies and those directed against PGI synthase and PGD synthase are compared in Table 11. IgG(162-24) stained only smooth muscle cells in kidney, heart and liver and failed to stain renal collecting tubules. Currently, IgG(162-26) showed immunofluorescence staining of proximal tubules and undifferentiated glomerular structures in human kidney (Table 11). These findings were surprising since (a) smooth muscle in large arteries forms the intima and media, (b) undifferentiated glomerular structures are present in the cortical zone of PGI (12, 43), and (c) proximal tubules have no detectable PGI synthase catalytic activity (121) or immunoreactivity (43).

### TABLE 11. Distribution of PGH-PGE Isomerase, PGI Synthase and PGD Synthase Immunoreactivities in Various Tissues

| First Antibody | Rat Kidney | Rabbit Kidney | Mouse Kidney | Rhesus Monkey | Sheep Vascular Gland |
|----------------|------------|---------------|--------------|---------------|---------------------|
| IgG(162-24)    | none       | none          | none         | none          | none                |
| IgG(162-25)    | vascular smooth muscle | vascular smooth muscle | vascular smooth muscle | vascular smooth muscle | vascular smooth muscle |
| IgG(162-26)    | none       | none          | proximal tubules, undifferentiated glomerular structures | intercellular staining between tubules | none |
| IgG(162-27)    | none       | collecting tubules, accessory fibrous septa, etc. | collecting tubules | collecting tubules | collecting tubules |

* indirect immunofluorescent staining of cryostat sections of fresh tissue was performed as described in the text.

* IgG(162-24) is reactive with rat PGI synthase (20, 33).

* IgG(162-25) also reacts with staining of epithelial cells of human's nasal, vascular smooth muscle and endothelial cells of normal rat kidney and of glomerular mesangial cells, vascular smooth muscle and endothelial cells of bovine kidney (43).