

Enzymes and Receptors of Prostaglandin Pathways with Arachidonic Acid-derived *Versus* Eicosapentaenoic Acid-derived Substrates and Products*[♦]

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Masayuki Wada^{‡1}, Cynthia J. DeLong^{‡1}, Yu H. Hong[‡], Caroline J. Rieke[‡], Inseok Song[‡], Ranjinder S. Sidhu[‡], Chong Yuan[‡], Mark Warnock[§], Alvin H. Schmaier^{§¶}, Chieko Yokoyama^{||}, Emer M. Smyth^{**}, Stephen J. Wilson^{**}, Garret A. FitzGerald^{**}, R. Michael Garavito^{‡‡}, De Xin Sui^{‡‡}, John W. Regan^{§§}, and William L. Smith^{‡2}

From the [‡]Department of Biological Chemistry and [§]Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan 48109, the [¶]Department of Medicine, Case Western Reserve University, Cleveland, Ohio 44106, the ^{||}21st Century Center of Excellence Program, Department of Cellular Physiological Chemistry, Tokyo Medical and Dental University, 1-5-45, Yushima, Tokyo-113-8549, Japan, the ^{**}Institute for Translational Medicine and Therapeutics, University of Pennsylvania, Philadelphia, Pennsylvania 19104, the ^{‡‡}Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, Michigan 48109, and the ^{§§}Department of Pharmacology and Toxicology, University of Arizona, Tucson, Arizona 85721

Dietary fish oil containing ω 3 highly unsaturated fatty acids has cardioprotective and anti-inflammatory effects. Prostaglandins (PGs) and thromboxanes are produced *in vivo* both from the ω 6 fatty acid arachidonic acid (AA) and the ω 3 fatty acid eicosapentaenoic acid (EPA). Certain beneficial effects of fish oil may result from altered PG metabolism resulting from increases in the EPA/AA ratios of precursor phospholipids. Here we report *in vitro* specificities of prostanoid enzymes and receptors toward EPA-derived, 3-series *versus* AA-derived, 2-series prostanoid substrates and products. The largest difference was seen with PG endoperoxide H synthase (PGHS)-1. Under optimal conditions purified PGHS-1 oxygenates EPA with only 10% of the efficiency of AA, and EPA significantly inhibits AA oxygenation by PGHS-1. Two- to 3-fold higher activities or potencies with 2-series *versus* 3-series compounds were observed with PGHS-2, PGD synthases, microsomal PGE synthase-1 and EP1, EP2, EP3, and FP receptors. Our most surprising observation was that AA oxygenation by PGHS-2 is only modestly inhibited by EPA (*i.e.* PGHS-2 exhibits a marked preference for AA when EPA and AA are tested together). Also unexpectedly, TxA₃ is about equipotent to TxA₂ at the TP α receptor. Our biochemical data predict that increasing phospholipid EPA/AA ratios in cells would dampen prostanoid signaling with the largest effects being on PGHS-1 pathways involving PGD, PGE, and PGF. Production of 2-series prostanoids from AA by PGHS-2 would be expected to decrease in proportion to the compensatory decrease in the AA content of phospholipids that would result from increased incorporation of ω 3 fatty acids such as EPA.

North American and Western European diets have relatively high levels of ω 6 fatty acids (*e.g.* linoleic acid (1, 2)). As a result, the most common highly unsaturated fatty acid is the C20 ω 6 fatty acid, arachidonic acid (AA).³ AA is present mainly at the *sn*2-position of membrane phospholipids. Humans ingesting fish oil enriched in ω 3 fatty acids show increased amounts of eicosapentaenoic acid (EPA) in their membrane phospholipids and an approximately corresponding decrease in the level of AA. The ratio of ω 3 EPA/ ω 6 AA in tissue phospholipids from human populations averages less than 0.1 (1, 2) but can be increased to almost 0.7 with palatable diets enriched in fish oil (3, 4). An increased dietary intake of fish oil is cardioprotective, anti-inflammatory, and anti-carcinogenic (2, 5–14).

The molecular basis for the health benefits of dietary fish oil is almost surely multifactorial. For example, ω 3 fatty acids attenuate responses of T-cells (15) and macrophages (16) to agents working through cell surface receptors perhaps by changing the composition of membrane microdomains (17, 18). One ω 3 fatty acid, docosahexaenoic acid (DHA), has been shown to be essential in the development and maintenance of neuronal functions including visual acuity. This may also be related to the ability of DHA to change the physical properties of membranes in a way that facilitates rhodopsin signaling (17, 19–22). Anti-arrhythmic effects of ω 3 fatty acids may relate to their stabilizing effect on cardiac cell membranes and inhibition of the fast, voltage-dependent sodium and L-type calcium currents (12). Nonesterified polyunsaturated fatty acids, particularly EPA, can also influence transcription acting through peroxisomal proliferator-activated receptors and sterol response

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¹ These authors contributed equally to this work.

² To whom correspondence should be addressed: Dept. of Biological Chemistry, University of Michigan Medical School, 5301 Medical Sciences Research Bldg. III, 1150 W. Medical Center Dr., Ann Arbor, MI 48109-0606. Tel.: 734-647-6180; Fax: 734-764-3509; E-mail: smithww@umich.edu.

³ The abbreviations used are: AA, arachidonic acid; PG, prostaglandin; PGDS, PGD synthase; mPGES-1, microsomal PGE synthase-1; PGHS, prostaglandin endoperoxide H synthase; COX, cyclooxygenase; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; PRP, platelet-rich plasma; Tx, thromboxane; TxAS, thromboxane A synthase; HHTe, 12(S)-hydroxy-5,8,10-heptadecatrienoic acid; HHTe, 12(S)-hydroxy-5,8,10-heptadecatetraenoic acid; hu, human; mu, murine; ov, ovine; IP, inositol phosphate; YPD, yeast extract/peptone/dextrose; YEL, yeast extract-sodium lactate; DMEM, Dulbecco's modified Eagle's medium; PBS, phosphate-buffered saline; Ni-NTA, nickel-nitrilotriacetic acid; MES, 4-morpholineethanesulfonic acid.

element binding protein-1c, major transcription factors controlling lipid metabolism (2, 23, 24). Other proteins that can be activated directly by polyunsaturated fatty acids, and thus, whose activities might be altered by changes in EPA/AA ratios include protein kinase C (25), NADPH oxidase (26), and a two-pore domain K^+ channel (27). Polyunsaturated fatty acids such as AA can promote apoptosis but the mechanism is not known (28).

Finally, the eicosanoid pathways for lipid mediator formation; including the cyclooxygenase pathways, the 5-, 12-, and 15-lipoxygenase pathways, the P450 epoxygenase pathways, and non-enzymic oxidative pathways; are influenced by changes in EPA/AA ratios (29–35). Anti-thrombotic, anti-inflammatory, and anti-carcinogenic effects of ω 3 fatty acids could result, at least in part, from their ability to attenuate the synthesis of specific eicosanoids and/or to alter the nature of the eicosanoid products formed or to serve as precursors of novel products such as isoprostanes and resolvins (32, 33, 35–38).

Prostanoids are synthesized via the cyclooxygenase pathway, most commonly from AA, in response to various hormones and physical stimuli (29). The pathway involves three stages: (a) mobilization of AA from membrane phospholipids by cytosolic phospholipase $A_2\alpha$ (cPLA $_2\alpha$) sometimes in conjunction with secretory sPLA $_2$ s; (b) conversion of AA to the prostaglandin endoperoxide PGH $_2$ by prostaglandin endoperoxide H synthase-1 or -2 (PGHS-1 or -2) also known as cyclooxygenase-1 or -2 (COX-1 or -2); and (c) isomerization of PGH $_2$ to a “2-series” product, PGD $_2$, PGE $_2$, PGF $_{2\alpha}$, PGI $_2$, or thromboxane A $_2$ (TxA $_2$), by specific synthases. Newly formed PGs exit cells and function primarily through G-protein-coupled receptors on neighboring or parent cells to elicit responses. Because PGs act at or near their sites of synthesis and are rapidly metabolized, they are considered to be “local” hormones. Importantly, EPA can serve as a substrate for PG formation generating “3-series” PG products including PGD $_3$, PGE $_3$, PGF $_{3\alpha}$, PGI $_3$, and TxA $_3$.

There is only limited biochemical information available on the specificities of the enzymes and receptors of the prostanoid pathways with EPA-derived *versus* AA-derived substrates and products. Here we report studies that address this topic.

EXPERIMENTAL PROCEDURES

Materials—U46619, Δ^{17} U46619, PGI $_2$, PGI $_3$, iloprost, SQ29548, AA, EPA, HHTrE, lipocalin PGD synthase (L-PGDS), and hematopoietic (H) PGDS were purchased from Cayman Chemicals (Ann Arbor, MI). [3 H]SQ29548 was purchased from PerkinElmer Life Sciences. [14 C]AA and [14 C]EPA were from American Radiolabeled Chemicals. [3 H]myoinositol and a cAMP assay kits were from Amersham Biosciences. SQ22536 was from Biomol. Cell culture materials were purchased from Invitrogen. Human fibrinogen, α -thrombin, and γ IIa-thrombin were purchased from Hematologic Technologies, Inc. Collagen was obtained from Chronolog Corp. Complete protease inhibitor was from Roche Applied Science. BCA protein reagent was from Pierce. Restriction enzymes were from New England Biolabs, Inc. Ni-NTA was from Qiagen. All other materials were purchased from Fisher Scientific.

Expression, Purification, and Assay of PGHSs—Hexahistidine-tagged (His $_6$) ovine (ov) PGHS-1, murine (mu) murine PGHS-2, and human (hu) PGHS-2 were expressed in S21 insect cells and purified through Ni-NTA chromatography essentially as described previously (39–41). His $_6$ -muPGHS-1 was expressed also in insect cells but was unstable following Ni-NTA chromatography so in the experiment using this enzyme, the supernatant from centrifugation of solubilized cell pellets was used for COX assays. Oxygen electrode assays for COX activity were performed as detailed in previous reports (39–41). COX assays of purified enzymes utilizing radio thin layer chromatography assays of PGH $_2$ or PGH $_3$ formation were performed using [14 C]AA and [14 C]EPA as described previously (41).

Preparation of Platelet-rich Plasma (PRP)—Platelets were obtained from normal human donors who had not taken medication during the 2 weeks prior to donation. Whole blood was drawn into 3.8% sodium citrate (1:9; citrate: blood). The blood was centrifuged at $180 \times g$ for 10 min at room temperature, and PRP was transferred to a new tube. The remaining blood was centrifuged at $1000 \times g$ for 10 min at room temperature to obtain PRP. For aggregation studies using PRP, the platelet count was determined on a Coulter counter (Model Z; Coulter Electronics, Hialeah, FL) and adjusted with HEPES-Tyrodé's buffer (137 mM NaCl, 3 mM KCl, 12 mM NaHCO $_3$, 0.34 mM Na $_2$ HPO $_4$, 14.7 mM HEPES, 0.35% dextrose, and 0.35% bovine serum albumin, pH 7.4) to 2.2 to 2.5×10^8 platelets/ml. For preparation of washed platelets, human platelets in PRP were separated from plasma by gel filtration over Sepharose 2B columns in HEPES-Tyrodé's buffer. The peak tubes were pooled, and the platelet count was adjusted to 2.5×10^8 platelets/ml before proceeding with platelet aggregation studies. Washed platelets (400 μ l) were placed in a cuvette in the aggregometer and stirred at 37 °C. The integrity of the washed platelets was tested by their ability to be activated by collagen (1–5 mg/ml) and α -thrombin (3 nM).

PGDS Assays—PGDS activity was determined essentially as described previously (42, 43). First, PGH $_2$ or PGH $_3$ were prepared from 18 μ M [14 C]AA or [14 C]EPA, respectively, by incubation for 20 s at room temperature with purified His $_6$ -muPGHS-2 (30 unit) in 100 μ l of 0.1 M Tris-Cl, pH 8.0, containing 2 mM phenol, 20 μ M hematin, and γ -globulin (1 mg/ml). PGH $_2$ /PGH $_3$ isomerization to PGD $_2$ /PGD $_3$ was initiated by the addition of either lipocalin or hematopoietic PGDS (0.04 unit) premixed with 0.1 mM GSH, followed by incubation for 40 s at room temperature. Reactions were quenched by adding 500 μ l of diethylether/methanol/0.2 M citric acid (30:4:1). After vortexing for 10 s, the reaction mixture was centrifuged at $1000 \times g$ for 10 min at 4 °C. An aliquot of organic extract (100 μ l) was separated by thin layer chromatography on a silica gel plate in ethyl acetate/2,2,4-trimethylpentane/acetic acid/water (110:50:20:100). Regions of the plates migrating with PGD, PGH, and other products were scraped into vials and radioactivity quantified by liquid scintillation counting. One unit of PGDS enzyme represents 1 μ mol of PGD $_2$ /min at 25 °C in 100 mM Tris-HCl, pH 8.0, containing 1 mM GSH, 1 mg/ml γ -globulin, and 40 μ M PGH $_2$.

Expression, Purification, and Assay of Human Microsomal PGE Synthase-1 (hu mPGES-1)—The human PGES cDNA (Invitrogen) was amplified using High Fidelity PCR kit (Invitrogen) with the 5'-primer (with BspHI) GAA TTC ATC ATG ATC CCT GCC CAC AGC CTG GTG A and the 3'-primer (with HindIII and His₆-tag) CAT CCA AGC TTG TCA GTG GTG GTG GTG GTG CAG GTG GCG GGC CGC AAC. The PCR product was purified with a QIAquick PCR purification kit (Qiagen). The pRMGsp expression vector⁴ was digested by AflIII and XhoI and the amplified PGES PCR product was digested with BspHI and HindIII. The digested DNA was isolated by electrophoresis on a 1% agarose gel, and the DNA band was purified with a QIAquick gel purification kit (Qiagen). The digested and purified expression plasmid and His₆-hu mPGES-1 insert were ligated with T4 DNA ligase. The ligation sample was transformed into DH5- α competent cells, and plasmids from positive colonies were sequenced to confirm the expression construct pRMGsp-PGES-His₆.

The pRMGsp-PGES-His₆ DNA with the aid DNA pAL9 (digested by PstI) were transformed into freshly made *Schizosaccharomyces pombe* competent cells. After selection of positive colonies on MAA plates, the potential transformants were screened twice on yeast extract/peptone/dextrose medium (YPD)/G418 plates (containing G418 at 20 μ g/ml). Positive single colonies were then grown in yeast extract-sodium lactate (YEL) medium (with 10 μ g/ml G418) to make glycerol stocks, which were stored at -80°C .

For expression of His₆-hu mPGES-1 in *S. pombe*, 5 ml of YEL medium (with 10 μ g/ml G418) was inoculated with 200 μ l of a glycerol stock culture and shaken for 24 h at 32°C . The culture was transferred into 50 ml of fresh YEL/G418 medium and incubated at 32°C for 48 h with shaking until the A_{600} was 10–12. The culture was then transferred into 1 liter of YPD medium (with 100 μ g of G419/ml) and incubated at 32°C with shaking until the A_{600} exceeds 20 (~ 48 –60 h). The cells were harvested by centrifugation at $3000 \times g$ for 15 min at 4°C , and the cell pellet was stored at -80°C .

After completely thawing the cell paste in ice water, 5 ml of lysis buffer (15 mM Tris-HCl, 250 mM sucrose, 0.1 mM EDTA, 1 mM reduced glutathione, pH 8.0) was added for per gram of cell pellet. The resuspended cell pellet was lysed using an Emulsi-Flex-C3 (at 20,000–25,000 p.s.i. with two passes). The cell lysate was centrifuged at $8000 \times g$ for 20 min at 4°C . The membrane-containing supernatant fraction was then centrifuged at $\sim 200,000 \times g$ for 1 h at 4°C . The membrane pellet was resuspended in loading buffer (50 mM sodium phosphate, 300 mM NaCl, 10% glycerol, pH 8.0) and dodecyl maltoside (Anatrace) was added to a final concentration of 1%. The membrane fraction was stirred for 1 h at 4°C and then centrifuged at $200,000 \times g$ for 1 h at 4°C . Imidazole was added to the supernatant fraction to a final concentration of 10 mM, and the mixture was loaded onto an Ni-NTA (Qiagen) column equilibrated with loading buffer supplemented with 10 mM imidazole; all column buffers contained 0.05% dodecyl maltoside. After loading, the column was washed with loading buffer containing 22 mM

imidazole. The bound His₆-hu mPGES-1 was eluted using loading buffer containing 200 mM imidazole. Elution fractions containing His₆-hu mPGES-1 were pooled and concentrated using a 30 molecular weight cutoff Centricon spin concentrator.

Microsomal Preparations of TxA Synthase (TxAS)—Mouse (mu) TXAS was expressed in Sf21 insect cells as described previously (44, 45). The cells from a 250-ml culture were harvested after 4 days, collected, and washed twice with ice-cold phosphate-buffered saline (PBS) and stored at -80°C . Cell pellets were thawed on ice and resuspended in 100 mM Tris-HCl pH 7.4, 1 mM EDTA and $1\times$ Complete protease inhibitor. Cells were disrupted by sonication and centrifuged at $10,000 \times g$ for 10 min at 4°C . The supernatant was then centrifuged at $100,000 \times g$ for 1 h at 4°C . The resulting pellet was homogenized in 10 mM Tris-HCl, pH 7.4, 0.1 mM EDTA, and 20% glycerol using a Dounce homogenizer and the protein concentration measured. The protein was used immediately for *in vitro* synthesis of TxA₂ or TxA₃.

PGE (EP), PGF (FP), and TxA/PGH (TP) Receptor Binding—HEK cell lines expressing various human PGE (EP2, EP3 (EP3II isoform (46)), EP4 (47, 48)), PGF (FP; (49)), PGI (IP; (50)), and TxA/PGH (TP; (51, 52)) receptors were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat inactivated fetal bovine serum, 250 μ g/ml Geneticin, 200 μ g/ml hygromycin, 100 μ g/ml gentamicin and maintained at 37°C with 5% CO₂. The cells were grown to 60% confluence and harvested from five 100-mm tissue culture dishes by scraping into the medium and centrifuged at $100 \times g$ for 5 min. The cell pellets were washed once with ice-cold PBS, harvested, and stored at -80°C until membranes were prepared for competitive binding assays.

The PCR was used to amplify the coding domain of the huEP1 receptor (nucleotides 1–1209; GenBankTM accession number L22647 (53)) from human kidney cDNA. The product encoding the huEP1 was purified by agarose gel electrophoresis and cloned into the EcoRV site of pcDNA3 to yield huEP1/pcDNA3. The sequence of huEP1 in huEP1/pcDNA3 was verified by DNA sequencing. huEP1/pcDNA3 encoding the huEP1 was transiently transfected into HEK293 cells using Lipofectamine 2000 according to the recommendation of the manufacturer, and cells were harvested 30 h post-transfection. Cell pellets were stored at -80°C and membranes prepared from these cells were used to determine the relative affinities of PGE₂ versus PGE₃.

Membranes were prepared from HEK293 cells essentially as described by Ungrin *et al.* (54). Briefly, cell pellets were thawed on ice and resuspended in Buffer A (10 mM HEPES/KOH, pH 7.4, with 1 mM EDTA and $1\times$ Complete protease inhibitor), disrupted by sonication, and centrifuged at $10,000 \times g$ for 10 min at 4°C . The supernatant was centrifuged at $100,000 \times g$ for 1 h at 4°C . The pellet was homogenized in Buffer A, and aliquots of the suspended protein (50–100 μ g) were used immediately for binding assays.

Binding assays were performed in 200 μ l of 10 mM MES, pH 6.0, 1 mM EDTA and 10 mM MgCl₂. Binding isotherms were performed for [³H]PGE₂, [³H]PGF_{2 α} , or the TPA antagonist [³H]SQ29548 to estimate K_d values for the different receptors with the cognate 2-series PG ligand. A concentration of ³H-la-

⁴ D. Sui and R. M. Garavito, unpublished results.

beled ligand corresponding to the K_d value for each receptor was then used in competition binding assays with PGE₂ versus PGE₃, PGF_{2α} versus PGF_{3α}, or U46619 versus Δ^{17} U46619. Non-specific binding was determined in the presence of 10 μ M unlabeled ligand. Samples were incubated at 30 °C for 1 h and then filtered through Whatman GF/C glass filters. The filters were washed three times with cold MES buffer (without EDTA) and radioactivity measured by liquid scintillation counting. Receptor binding data were analyzed by nonlinear regression in Origin. Statistical analyses were performed using Student's *t* test and/or ANOVA.

cAMP Assays—HEK cell lines expressing EP2 and EP4 receptors that had been grown as described above in 6-well plates were treated for 15 min at 37 °C with fresh DMEM containing 50 mM isobutylmethylxanthine. Cells were then treated with various concentrations of PGE₂ or PGE₃ for an hour. The treatments were terminated by scrapping cells into 0.5 ml of TE (50 mM Tris-HCl, pH 7.5, containing 4 mM EDTA) and boiling for 8 min. After centrifuging the lysates, 50 μ l of the supernatant (from about 10⁵ cells) was used for cAMP analysis using an Amersham Biosciences cAMP assay system kit following the instructions of the manufacturer. The samples were quantified by scintillation counting and values for cAMP were calculated from the cAMP standard curve.

Assays of Inositol Phosphates (IPs)—Assay of IPs was performed by measuring receptor induced production of [³H] IPs as described previously (55). Briefly, HEK293 cells expressing EP3 or FP receptors were grown in 24-well plates as described above and labeled by incubating overnight with 1 μ Ci of [³H]myoinositol (Amersham Biosciences) per ml of DMEM. The cells were treated with 10 mM LiCl for 15 min prior to adding various concentrations of PGE₂/PGE₃ or PGF_{2α}/PGF_{3α} for 1 h. Assays were terminated by adding 3 ml of chloroform/methanol/water (1:1:1) to each well. The whole cell lysates were collected and centrifuged, and the resulting aqueous phase was applied to a Dowex AG1-X8 anion exchange column (Bio-Rad) to remove unincorporated [³H]myoinositol. The IPs were eluted with 0.2 M ammonium formate, 0.1 M formic acid and quantified by liquid scintillation counting.

Measurements of Intracellular Ca²⁺—Intracellular Ca²⁺ concentrations were measured as described previously by Fisher *et al.* (56). Nontransfected HEK293 cells and huEP1 plasmid-transfected HEK293 cells were incubated with 1 μ M Fura-2/AM (Invitrogen) for 15 min before adding various concentrations of PGE₂ or PGE₃. The Ca²⁺ signals were measured using a Shimadzu RF-5301 PC spectrofluorometer.

Platelet Aggregation—Aggregation assays were performed on a Chronolog dual-channel aggregometer at 37 °C. The assays were carried out with 360 μ l of undiluted human PRP, and the compounds to be tested were added to obtain a final volume of 400 μ l. The assay was measured for 3 min following platelet activation. Only PRP that was responsive to 20–40 nM γ -Ila-thrombin was used for experiments. Variable amounts of each aggregatory compound were added to PRP to determine the threshold concentration for platelet aggregation: U46619 or Δ^{17} U46619 (0.1 nM to 2 μ M) and collagen (1–2 μ g/ml). In some experiments, inhibition of aggregation induced by 2 μ M U46619 was examined using different amounts of anti-aggre-

gatory compounds: iloprost (0.1–10 nM) and PGI₂ or PGI₃ (10 pM to 2 μ M); flurbiprofen was added at 100 μ M. All compounds were diluted from a stock solution in organic solvent into PBS, pH 7.5, prior to the collection of blood, except for PGI₂ and PGI₃, which were prepared by removing a solid chemical stock vial from –80 °C (stored for less than 1 week) and dissolving in PBS less than 15 min before adding to PRP. The stability of each prostacyclin was checked by comparing the anti-aggregatory activity of 1 nM at the end of the experiment to that of the beginning of the experiment.

Platelet Aggregation by TxA₂ Versus TxA₃—An aliquot of PRP (370 μ l) was placed into a cuvette in the aggregometer while stirring at 37 °C. The TxA₂ and TxA₃ were prepared just before adding to PRP. In an Eppendorf tube, AA or EPA was added to a final concentration of 5 μ M in reaction buffer (100 mM Tris-HCl, pH 7.4, 1 mM phenol, and 10 μ M heme) containing 260 units of COX-2 to initiate the synthesis of PGH₂ or PGH₃. The sample was vortexed for 20 s. Then, 30 μ l of TxA microsomal protein (~500 μ g) was added to produce TxA₂ or TxA₃ in a final volume of 100 μ l. The mixture was vortexed for 10 s, at which time 30 μ l was removed and added to the 370 μ l of PRP. Immediately, prior to the experiments using PRP, parallel reactions were performed with ¹⁴C-labeled AA or EPA and the products quantified by radio thin layer chromatography as described above. This provided an accurate estimate of the various products being added in each case to the PRP.

RESULTS

Specificities of PGHS-1 and PGHS-2 with AA and EPA—As shown in Fig. 1, purified ovPGHS-1 and muPGHS-2 oxygenate AA with comparable catalytic efficiencies at concentrations of ≥ 1 μ M AA where reasonably precise O₂ electrode measurements of enzyme activity can be performed. ovPGHS-1 is essentially inactive with EPA while muPGHS-2 can use EPA with about 30% of the efficiency of AA in the range of 1–100 mM.⁵ A solubilized preparation of muPGHS-1 expressed in baculovirus showed qualitatively similar results to those shown for ovPGHS-1 (data not shown); muPGHS-1 was unstable in our hands, and so we were unable to analyze purified enzyme. Although ovPGHS-1 was not active with low concentrations of EPA, significant activity (~10% of that with AA) was observed when 15 μ M 15-hydroperoxyeicosatetraenoic acid was added to the reaction mixtures (data not shown). Purified huPGHS-2 also showed results very similar to those in Fig. 1 for muPGHS-2 (data not shown) when tested under essentially identical enzyme and substrate conditions.

Results similar to those illustrated in Fig. 1 have been reported by Kulmacz and co-workers using ovPGHS-1 (57, 58). Moreover, the results with purified and semipurified enzymes are consistent with studies comparing the utilization of AA versus EPA by microsomal huPGHS-1 and huPGHS-2 (59), where, under optimal conditions, huPGHS-1 is 5% as active with EPA as with AA while huPGHS-2 is 25–30% as active with EPA as

⁵ Critical micelle concentrations for AA and EPA determined in 0.1 M sodium phosphate, pH 7.6, using fluorescence assays (*i.e.* with 1 mM *N*-phenyl-1-naphthylamine) were approximately 62 and 210 mM, respectively.

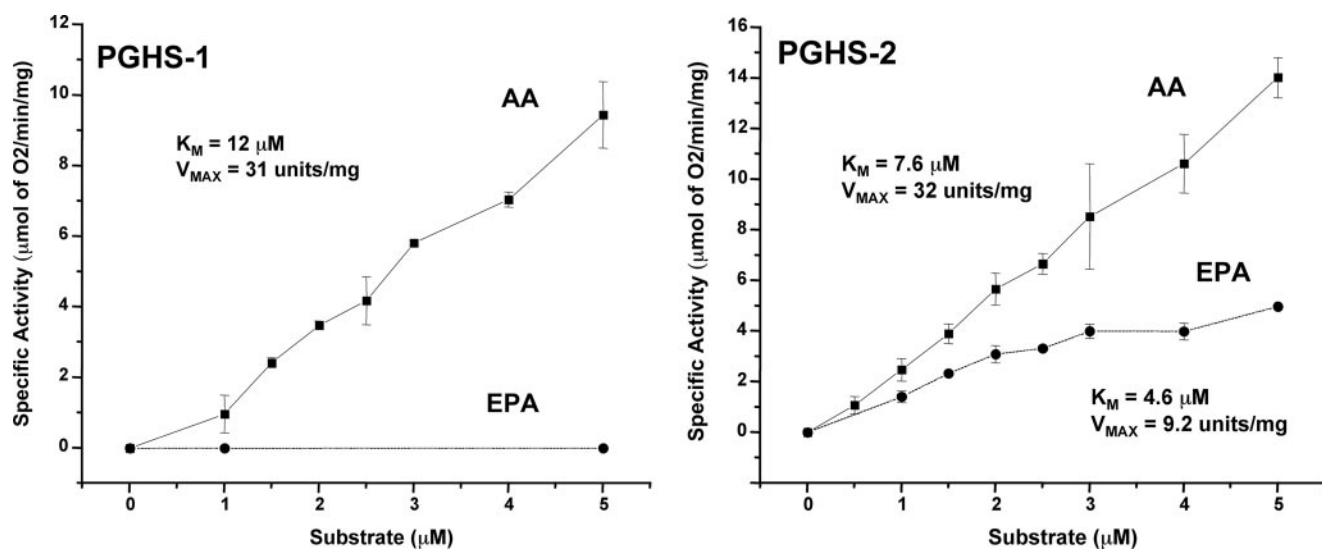


FIGURE 1. **COX activities of ovPGHS-1 (left panel) and muPGHS-2 (right panel) with AA and EPA.** Specific activities were measured using purified His₆-tagged native ovPGHS-1 (~8 μg) or His₆-tagged muPGHS-2 (ca. 8 μg). Assays were performed on an O₂ electrode using standard COX assays with the indicated concentrations of substrates.⁵ Shown in this figure are results obtained with fatty acid substrate concentrations of 1–5 μM ; however, K_m and V_{max} values were determined using fatty acid substrate concentrations of 1–100 μM . Circles, AA; squares, EPA. Each data point represents a total of four assays involving two separate enzyme preparations, and error bars represent mean \pm S.D.

AA. It also is clear that EPA can be oxygenated by PGHS-1 in intact cells in a manner that is peroxide dependent (60).

EPA and AA have similar K_m values with PGHS-1 and PGHS-2 (Fig. 1 and (57, 59)), and so EPA would be expected to compete with AA for oxygenation. EPA/AA competition has been shown previously with PGHS-1 (61), and the results in Fig. 2 confirm these findings. Half-maximal inhibition occurs with equimolar AA *versus* EPA. Essentially identical results were also obtained with a solubilized preparation of His₆-muPGHS-1. AA is about a 10 times better substrate than EPA for ovPGHS-1 *in vitro*, and as shown in Fig. 3, inhibition of oxygenation reflects primarily inhibition of AA oxygenation. A 5-fold excess of EPA caused 40% inhibition of [1-¹⁴C]AA oxygenation by ovPGHS-1. This result is similar but not identical to that of Fig. 2, which shows about 75% inhibition at these concentrations of AA plus EPA. As expected, [1-¹⁴C]EPA was a poor substrate for PGHS-1; however, EPA oxygenation was augmented slightly by the presence of AA. Again, this is probably because hydroperoxide is being generated when AA is present along with EPA in the reaction mixtures and hydroperoxides potentiate EPA oxygenation (59, 60, 62).

In contrast to the results obtained with PGHS-1, EPA was a relatively poor inhibitor of AA oxygenation by PGHS-2 (Fig. 2). For example, at equimolar AA and EPA concentrations, the first point at which there was a statistically significant decrease in the rate of oxygenation with muPGHS-2, there was only a 10% decrease in O₂ consumption and even with a 5-fold excess of EPA there was less than a 20% decrease in oxygenase activity. Based on the kinetic constants for muPGHS-2 (Fig. 1) and huPGHS-2 for AA and EPA tested individually, one would expect about a 35% lower oxygenation rate with 20 μM AA plus 20 μM EPA and a 60% decrease in the rate with 20 μM AA plus 100 μM EPA. To examine this inconsistency, we incubated purified enzymes with [1-¹⁴C]AA or [1-¹⁴C]EPA with and without unlabeled competing substrate and measured the formation of radioactive PGH₂ or PGH₃ (plus HHTE (12(*S*)-hydroxy-5,8,10-

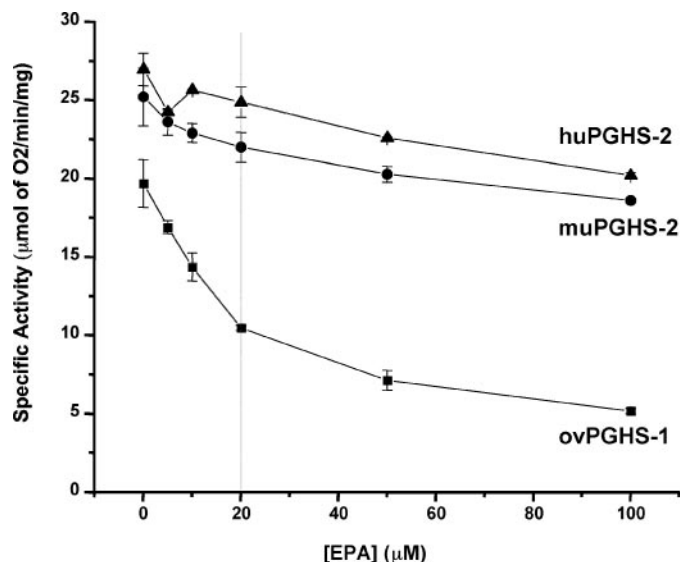


FIGURE 2. **Inhibition of COX activities of ovPGHS-1, muPGHS-2, and huPGHS-2 with AA and EPA.** Enzyme activity was measured using a standard COX oxygen electrode assay with 6–8 μg of His₆-tagged purified proteins, 20 μM AA, and the indicated concentrations of EPA as described under "Experimental Procedures" (39). Duplicate samples were assayed, and experiments with three different enzyme preparations yielded similar results. Error bars represent mean \pm S.D.

heptadecatetraenoic acid) a degradation product of PGH₃) using radio thin layer chromatography (Fig. 3). When 20 μM unlabeled AA was added to reaction mixtures containing 20 μM [1-¹⁴C]EPA, oxygenation of EPA by PGHS-2 was inhibited by 70–90% (Fig. 3). In contrast, with 20 μM [1-¹⁴C]AA and 100 μM unlabeled EPA there was only a modest inhibition (~10%) of AA oxygenation (Fig. 3, lower panel).

Thus, EPA acts as an effective inhibitor of AA oxygenation by PGHS-1 but not PGHS-2, and indeed PGHS-2 shows a marked and unanticipated preference for AA when presented with a mixture of AA and EPA. The basis for the uneven competition between AA and EPA with PGHS-2 is not clear. It may involve

half of sites activity with PGHS-2 (40). In the case of PGHS-2 but not PGHS-1, binding of certain fatty acids to one COX site may facilitate oxygenation of AA bound to the other site.

In brief, our results with PGHSs show that (a) AA is an equally good substrate for PGHS-1 and PGHS-2; (b) EPA is a poorer substrate than AA for both PGHS-1 and PGHS-2 and a particularly poor substrate for PGHS-1; (c) EPA is an efficient inhibitor of AA oxygenation by PGHS-1 but not PGHS-2; and

(d) in the presence of EPA, PGHS-2 shows a marked preference for AA.

Specificities of Lipocalin PGDS, Hematopoietic PGD Synthase (hPGDS), and Microsomal PGES-1 (mPGES-1) toward PGH₂ Versus PGH₃—Table 1 shows data obtained in estimating the specificities of hPGDS, lPGDS, and mPGES-1 with PGH₂ versus PGH₃. PGH₂ and particularly PGH₃ are unstable, and so they were generated *in situ* quantitatively from AA or EPA using an excess of purified PGHS-2 and then a PGDS or PGES was added immediately and the reactions continued for 20–40 s. PGH₃ was found to be less stable than PGH₂, so it was necessary to add more EPA than AA in generating the endoperoxides so that the PGH₂ and PGH₃ concentrations were about the same when a PGDS or PGES was added. The reactions were terminated before 20% of the PGH was consumed, and the reactions were performed with amounts of enzyme that provided approximately linear product formation with time. Somewhat different V_{\max} and K_m values have been reported for each of the various PGD and PGE synthases we tested (63–68). Because of this and the technical difficulties associated with multiple assays with unstable substrates and limited amounts of enzymes, we elected to use an endoperoxide substrate concentration in the range of 5 μM for all of our assays, because as noted earlier,⁵ 5 μM PGH₂ or PGH₃ would likely be as high a concentration as would be encountered by a PGDS or PGES in an intact cell. With all these provisos, the human versions of H-PGDS, L-PGDS, and mPGES-1 were all more than 3-fold less active with PGH₃ than with PGH₂.

Quantitative data comparing the specificities of various prostanoid biosynthetic enzymes with AA versus EPA derived substrates is summarized in Table 2.

PGE and PGF Receptor Specificities—Membranes from HEK cell lines expressing various PGE (EP) and PGF (FP) receptors were used to determine the relative affinities of 2- versus 3-series PGE and PGF (Table 3). Except for the EP4 receptor, the affinity of each receptor was significantly greater for the 2-series than the 3-series PGs. The most dramatic difference was with the FP receptor, which had a 78-fold higher affinity for PGF_{2 α} than PGF_{3 α} .

Fig. 4 shows the potencies of 2- versus 3-series PGs in eliciting second messenger formation via the EP and FP receptors. Differences were significant with EP1, EP2, EP3, and FP receptors as determined by ANOVA for EP1 ($p < 0.001$), EP2 ($p <$

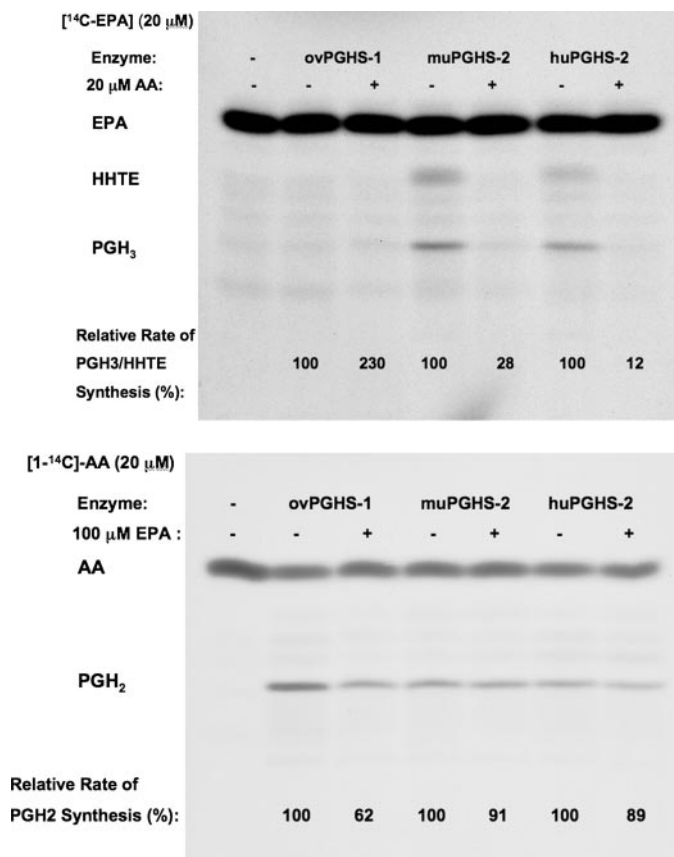


FIGURE 3. Oxygenation of [1-¹⁴C]EPA and [1-¹⁴C]AA in the presence and absence of unlabeled AA or unlabeled EPA. Radio thin layer chromatography assays were performed as described under "Experimental Procedures" (41). The indicated substrates were mixed with 0.5 μg (~ 12 units) of the purified His₆-tagged PGHSs and the reactions continued for 30 s. Products were extracted, separated, and visualized by autoradiography. The thin layer plates were subsequently scraped and the amounts of radioactivity associated with the substrates and products determined by scintillation counting and used to compute the relative rates indicated in the figure.

TABLE 1

Specificities of human hematopoietic and lipocalin PGD synthases and microsomal PGE synthase-1 toward PGH₂ versus PGH₃

Human hematopoietic PGD synthase (H-PGDS; 0.04 unit (1 μg /assay)) and lipocalin PGDS (L-PGDS; 0.04 unit (18 μg /assay)) PGD synthases were from Cayman Chemical Co. Purified, solubilized His₆ mPGES-1 was expressed and purified as indicated under "Experimental Procedures" and 0.67–1 μg used for the assays presented in the table. [1-¹⁴C]PGH₂ or [1-¹⁴C]PGH₃ was prepared by incubation of 18 μM [1-¹⁴C]AA or [1-¹⁴C]EPA for 20 or 40 s. Hematopoietic or lipocalin PGDS or mPGES-1 was then added and the incubations performed for 40 s for PGDSs or 20 sec for mPGES-1 under conditions in which the rate of conversion to product (PGD or PGE) was approximately linear with time and added enzyme. [1-¹⁴C]PGH₂ and [1-¹⁴C]PGH₃ were generated *in situ* with purified muPGHS-2 or huPGHS-2 and H-PGDS, L-PGDS, or PGES was added to initiate the reactions in a final volume of 0.1 ml. Products were extracted, separated at 4 $^{\circ}\text{C}$ by thin-layer chromatography, and quantified by scintillation counting. Values in parentheses are numbers normalized for the indicated starting PGH₂ concentration.

PG synthase	PGH ₂	PGH ₃	PGD ₂ or PGE ₂	PGD ₃ or PGE ₃	PGD ₂ /PGD ₃ or PGE ₂ /PGE ₃
	nmol	nmol	nmol		
H-PGDS (1 μg)	8.0	4.4 (8.0)	0.51	0.063 (0.11)	4.6
H-PGDS (1 μg)	3.8	3.8	0.26	0.043	6.0
H-PGDS (1 μg)	3.8	4.7 (3.8)	0.21	0.035 (0.028)	7.5
L-PGDS (18 μg)	3.8	4.7 (3.8)	0.056	0.021 (0.017)	3.3
mPGES-1 (0.67 μg)	6.0	3.2 (6.0)	0.13	0.021 (0.039)	3.2
mPGES-1 (1.0 μg)	4.4	4.5	0.069	0.025	2.7

TABLE 2

Specificities of PG biosynthetic enzymes with AA- versus EPA-derived substrates

Enzyme	AA-derived substrates		EPA-derived substrates		Ref.
	K_m	Rel. rates	K_m	Rel. rates	
cPLA ₂ sPLA ₂	$k_{cat}/K_m = 0.5$ Kinetic values are highly context dependent; mechanism of reaction does not permit discrimination among acyl groups.		Est. $k_{cat}/K_m = 0.5$ No activity without added hydroperoxide; <10% of activity with hydroperoxide; K_m similar to that of AA		74–76 79–81
ovPGHS-1	12 μM	31 units/mg	No activity without added hydroperoxide; <10% of activity with hydroperoxide; K_m similar to that of AA		57, 59, 83, 87; Fig. 1
muPGHS-2	7.6 μM	32 units/mg	4.6 μM	9.2 units/mg	57, 59; Fig. 1
H-PGDS	0.5 mM	$k_{cat} = 21 \text{ s}^{-1}$	17% activity with 5 μM PGH ₃ vs. PGH ₂		63, 64; Table 1
L-PGDS	14 μM	$k_{cat} \sim 50 \text{ s}^{-1}$	30% activity with 5 μM PGH ₃ vs. PGH ₂		65, 66; Table 1
mPGES-1	17 μM	$K_{cat} \sim 50 \text{ s}^{-1}$	30% activity with 5 μM PGH ₃ vs. PGH ₂		67, 68; Table 1
mPGES-2	28 μM	3.3 $\mu\text{mol}/\text{min}/\text{mg}$	ND		120
cPGES	14 μM	190 $\mu\text{mol}/\text{min}/\text{mg}$	ND		109
PGFS	Several enzymes proteins catalyzing the formation of PGF from PGH ₂ have been reported; it is not clear which are physiologically important.				103, 104
PGI synthase	30 μM	$k_{cat} \sim 5 \text{ s}^{-1}$	About the same activity with PGH ₃ and PGH ₂		45, 71
TxA synthase	22 μM	$k_{cat} = 27 \text{ s}^{-1}$	About the same activity with PGH ₃ and PGH ₂		71

TABLE 3

EP and FP receptor specificities for PGE₂ vs. PGE₃ and PGF_{2 α} vs. PGF_{3 α} Membranes were prepared from HEK293 cell lines that stably express the human EP2, EP3, EP4, and FP receptors essentially as described by Ungrin *et al.* (54) as detailed under "Experimental Procedures."

Ligand	IC ₅₀ × 10 ⁻⁹ M for ligand binding to receptor				
	EP1	EP2	EP3	EP4	FP
PGE ₂ or PGF _{2α}	15 ± 6.2 ^a	5.3 ± 0.86 ^a	7.7 ± 1.6 ^a	4.9 ± 1.4	2.3 ± 0.70 ^a
PGE ₃ or PGF _{3α}	110 ± 31	20 ± 5.3	37 ± 8.7	17 ± 11	180 ± 110
Relative affinities (PG ₃ vs. PG ₂)	7.3	3.8	4.8	3.5	78

^a Denotes significant difference between 2- and 3-series as determined by Student's *t* test. All binding assays were performed with duplicate samples with at least three different membrane preparations.

0.005), EP3 ($p < 0.0014$), and FP ($p < 0.0001$); ANOVA indicated no difference with the EP4 receptor ($p < 0.054$). In all cases the differences in potencies were less than the differences in binding affinities. However, it is important to note that, with the possible exception of the EP4 receptor, 3-series PGs were partial agonists. Quantitative data on receptor potencies are summarized in Table 4.

TP α Receptor Specificity toward U46619 and Δ^{17} U46619—Using membranes from HEK293 cells expressing the huTP α receptor, we determined the equilibrium dissociation constant for the TP α antagonist [³H]SQ29548 to be 9 μM . Equilibrium competition binding assays with the PGH₂/TxA₂ and PGH₃/TxA₃ analogues U46619 and Δ^{17} U46619, respectively, were used to measure displacement of 5 μM [³H]SQ29548 (Fig. 5). The IC₅₀ values for U46619 and Δ^{17} U46619 were identical (*i.e.* 200 and 210 nM, respectively).

Comparison of U46619 and Δ^{17} U46619 Activation of Platelet Aggregation—PRP from human donors was treated with U46619 or Δ^{17} U46619 in amounts ranging from 0.1 to 2 μM to measure the potency of each compound in platelet aggregation (Fig. 6). The threshold concentrations ranged from 0.5 to 0.8 μM for U46619 and from 0.7 to 1 μM for Δ^{17} U46619 for platelets from four donors. For individual donors, the relative potencies of the compounds were similar, with the threshold concentration of Δ^{17} U46619 consistently around 1.2-fold greater than that of U46619. To confirm the specificities of the analogues for the TP receptor, the platelets were incubated first with TP

antagonist SQ29548 (1 μM) before addition of the diene or triene analogue (2 μM) or of collagen (2 $\mu\text{g}/\text{ml}$). SQ29548 completely blocked aggregation by either U46619 or Δ^{17} U46619 and inhibited collagen-induced aggregation by ~50% (data not shown).

Effects of TxA₂ Versus TxA₃ on Platelet Aggregation—Previous studies had suggested that TxA₃ was essentially inactive in platelet aggregation (69, 70), while our results with Δ^{17} U46619 suggested that TxA₃ would be pro-aggregatory. This assessment raised the possibility that the Δ^{17} U46619 analogue behaves differently than authentic TxA₃. Because TxAs have very short half lives, we developed a system for synthesizing TxA₂ or TxA₃, which could then be added immediately to platelets. In brief, AA or EPA were treated with excess huCOX-2 to convert the fatty acids quantitatively to their respective endoperoxides. Excess microsomal TxAS was then added to quantitatively convert PGH₂ or PGH₃ to TxA₂ or TxA₃, respectively; TxAS is reported not to discriminate between PGH₂ and PGH₃ (71). An aliquot of the reaction mixture was immediately added to PRP and platelet aggregation was monitored. The amounts of the various products formed from AA and EPA by huPGHS-2 and TxAS were monitored in parallel reactions using [1-¹⁴C]AA or [1-¹⁴C]EPA.

A representative experiment is shown in Fig. 7. Reaction 2, with a concentration of 78 nM TxA₂, induced irreversible aggregation. However, Reaction 4, which contained 45 nM TxA₃, caused only a small reversible aggregation. When added to

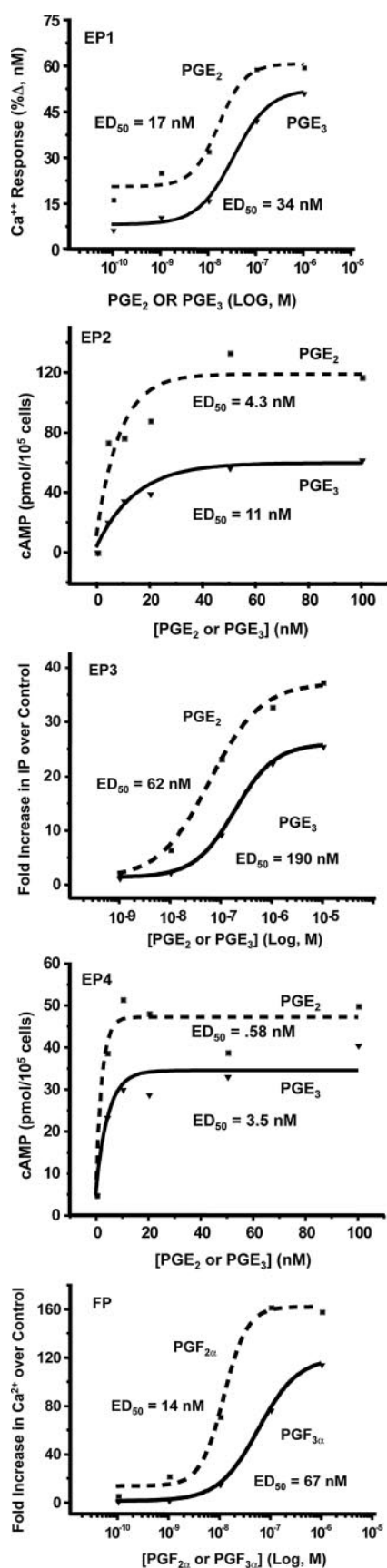


FIGURE 4. Potencies of 2- versus 3-series PGs in eliciting second messenger formation by various EP receptors and the FP receptor. HEK cells expressing the indicated EP1, EP2, EP3, EP4, and FP receptors were used to

human PRP, PGH_3 was found to isomerize to PGD_3 with a $t_{1/2}$ of 90 s, unlike PGH_2 which has a $t_{1/2}$ of 350 s. PGD_3 is also reported to be somewhat more potent at inhibiting aggregation than PGD_2 (70), both of which cause an increase in cyclic AMP. To block the putative inhibitory effects of any PGD_3 , 1 μM adenylylate cyclase inhibitor SQ22536 was added to the PRP prior to the addition of Reaction 5. This unmasked an aggregatory effect equal to that of the TxA_2 reaction (Reaction 2).

Both PGH_2 and PGH_3 are ligands of the TP receptor. However, neither the diene nor the triene endoperoxide produced in the PGHS-2-only reactions 1 and 3 (Fig. 7) was generated at sufficient concentrations to induce irreversible aggregation. Thus, residual endoperoxide in the reactions containing TxA_2 could not be responsible for the irreversible aggregation seen in Reactions 2 or 5.

Thromboxane and HHTrE are reported to be produced in equimolar amounts by TxA_2 (71). However, in our *in vitro* reaction, HHTrE and HTE were produced at approximately twice the concentration of their respective thromboxanes. The effect of HHTrE on platelet aggregation was investigated by adding 1 μM HHTrE to PRP alone or prior to the addition of either 2 μM U46619 or 2 $\mu\text{g/ml}$ collagen. HHTrE neither induced nor inhibited platelet aggregation (results not shown). Likewise, up to 100 μM malondialdehyde, another side product of the TxA_2 reaction, had no effect on platelet aggregation (results not shown). The results of studies with the TPa receptor and platelet aggregation suggest that TxA_2 or TxA_3 are approximately equipotent.

Comparison of PGI_2 and PGI_3 as Inhibitors of Platelet Aggregation— PGI_2 or PGI_3 (0.1–2 μM) was added to PRP, immediately followed by 2 μM U46619. Preliminary experiments with several donors were performed to optimize experimental conditions, including the stabilization of the prostacyclins and determination of the approximate threshold concentrations of each compound, before proceeding to perform dose response measurements with three donors (Fig. 8). The initial slope of each curve in Fig. 8 was measured and expressed as the percent inhibition of aggregation *versus* PGI concentration. The average IC_{50} values were 0.92 ± 0.28 nM and 1.30 ± 0.18 nM for PGI_2 and PGI_3 , respectively. Thus, the potencies of PGI_2 and PGI_3 in inhibiting platelet aggregation are approximately the same confirming earlier results (69).

DISCUSSION

The goal of the studies reported here was to compare the specificities and potencies of PG biosynthetic enzymes and receptors toward AA-derived, 2-series *versus* EPA-derived, 3-series substrates and products. We reason that this new information will contribute to understanding whether any of the reported beneficial health effects of dietary $\omega 3$ fish oil fatty acids are mediated through PG pathways. Our biochemical results along with those of others are summarized in Fig. 9 and Tables 2 and 4.

measure changes in cAMP or IP formation, or Ca^{2+} mobilization with the indicated concentrations of PGE_2 , PGE_3 , $\text{PGF}_{2\alpha}$ or $\text{PGF}_{3\alpha}$. Details of the experimental protocols are presented under "Experimental Procedures." All assays were performed in duplicate or triplicate with at least three cell preparations and data analyzed using ANOVA.

TABLE 4

PG receptors and their affinities or potencies with AA- vs. EPA-derived PGs

Receptor	EC ₅₀ 2-series PG	EC ₅₀ 3-series PG	Second messenger	Cell/Tissue	Ref.
	<i>nm</i>	<i>nm</i>			
DP1	109	64	G _s , cAMP	Platelets	70, 111
DP2	7	8	G _i , Ca ²⁺	Eosinophil	111
EP1	17	34 ^a	G _q , Ca ²⁺	EP1 HEK cell	Table 3, Fig. 4; PGE ₃ is partial agonist
EP2	4.3	11 ^a	G _s , cAMP	EP2 HEK cell	Table 3, Fig. 4; PGE ₃ is partial agonist
EP3	62	190 ^a	G _i , reduced cAMP, IP increases	EP3 HEK cell	Table 3, Fig. 4; PGE ₃ is partial agonist
EP4	0.58	3.5	G _s , cAMP	EP4 HEK cell	Table 3, Fig. 4; PGE ₃ is partial agonist
FP	14	67 ^a	G _q , Ca ²⁺	FP HEK cell	Table 3, Fig. 4; PGE ₃ is partial agonist
IP	0.92	1.3	G _s , cAMP	Platelets	Fig. 8
TP	650 for U46619	850 for Δ ¹⁷ -U46619	G _q , Ca ²⁺	Platelets	Fig. 6

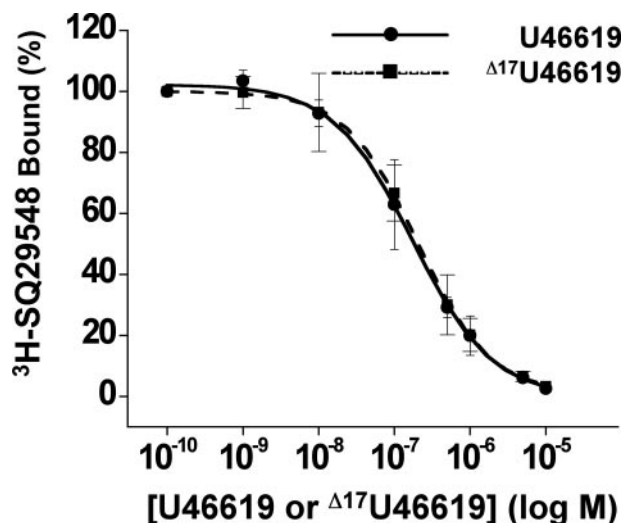
^a Denotes significant difference. ANOVA (*p* < 0.05).

FIGURE 5. **Binding of U46619 and Δ^{17} U46619 to the huTP α receptor.** An equilibrium competition binding assay was performed for U46619 and Δ^{17} U46619 versus [³H]SQ29548 (5 μ M) as the radioligand. The average K_d and B_{max} values for [³H]SQ29548 binding to TPA/HEK293 microsomes from three experiments was 10.2 ± 3.1 nM and 3800 ± 980 fmol/mg of protein, respectively. The average IC₅₀ values for U46619 and Δ^{17} U46619 were 200 and 210 nM, respectively, from an average of three experiments depicted in the figure.

Phospholipases—cPLA₂ α is the key phospholipase involved in AA release in most PG forming cells (72). Previous studies have shown that cPLA₂ α exhibits specificity toward AA and EPA esterified at the 2-position of phospholipids in comparison to other 2-position acids such as linoleate and oleate (73–76). Moreover, although EPA- and AA-containing phospholipids are equally good substrates for cPLA₂ α , DHA-containing phospholipids are essentially inactive with cPLA₂ α (76–78). Certain sPLA₂ forms can also participate in PG biosynthesis (79, 80). Because of the nature of the interaction with its substrates, sPLA₂ does not discriminate among 2-position acyl groups (81). In short, neither cPLA₂ nor sPLA₂ appears to differentiate between the acyl chains of AA versus EPA.

PGHS-1 and PGHS-2—PGHS-1 and PGHS-2 both exhibit specificity toward AA versus EPA. As reported previously by others and us, both enzymes have very similar K_m and V_{max} values with AA (57, 59). PGHS-2 oxygenates EPA at about 30% of the rate of AA. Purified PGHS-1 is not active with EPA unless an exogenous hydroperoxide is added to the reaction mixture. This is a consequence of the higher hydroperoxide requirement of PGHS-1 versus PGHS-2 (57, 82, 83). PGHS-1 present in platelets cells does oxygenate exogenously supplied EPA albeit at a low rate in the presence of alkyl hydroperoxides (60). It is

not clear whether the hydroperoxide concentration in cells is usually sufficient to support EPA oxygenation or whether there are differences in hydroperoxide concentrations among cell types (57, 84).

The behavior of PGHS-1 with AA plus EPA is consistent with the kinetic properties of the enzyme determined with AA and EPA individually; thus, EPA is a reasonably good inhibitor of AA oxygenation by PGHS-1 as was originally reported by Lands and co-workers (61). A comparison of the crystal structures of EPA and AA with PGHS-1 suggests that EPA prefers to bind in a catalytically incompetent conformation in the PGHS-1 cyclooxygenase site and competes with AA for binding (85–87).

PGHS-1 mediated biological events include platelet aggregation and parturition (88, 89) and certain types of acute inflammation (79, 90, 91). Cellular events involving PGHS-1 may be dampened when EPA/AA ratios in phospholipids are increased. At an EPA/AA ratio of 1.0, one would expect that there would be 50% less AA to be mobilized from phospholipids by cPLA₂ and that PGHS-1 would function at only 50% of maximal efficiency because of inhibition of AA oxygenation by EPA; however, this may well be an oversimplification because the concentrations of enzymes, receptors, and substrates in intact cells are unknown.

One of our most surprising observations was that PGHS-2 preferentially oxygenates AA when EPA and AA are tested together. The results observed when PGHS-2 is mixed with EPA plus AA cannot be explained based on the simple kinetic properties of PGHS-2 with AA or EPA individually. The biochemical basis for the selectivity of PGHS-2 for EPA versus AA when the substrates are together may relate to the half of sites activity of the enzyme (40). One possibility is that EPA binds one of the two cyclooxygenase sites of the PGHS-2 dimer and elicits an allosteric effect on the other cyclooxygenase site causing it to preferentially bind and oxygenate AA. If this is true and also applicable to any fatty acid, it could explain why PGHS-2 can preferentially oxygenate AA at low substrate concentrations when AA represents a small part of the available fatty acid pool in cells (92–94). A situation like this could occur in so-called late phase PG synthesis when an sPLA₂ is the operative phospholipase (95).

PGs are importantly involved in inflammation (79, 91, 96–98), and in this context PGHS-2 is the most important PGHS isoform (99, 100). Based on our biochemical studies, a decrease in the formation of 2-series PGs via PGHS-2 would be

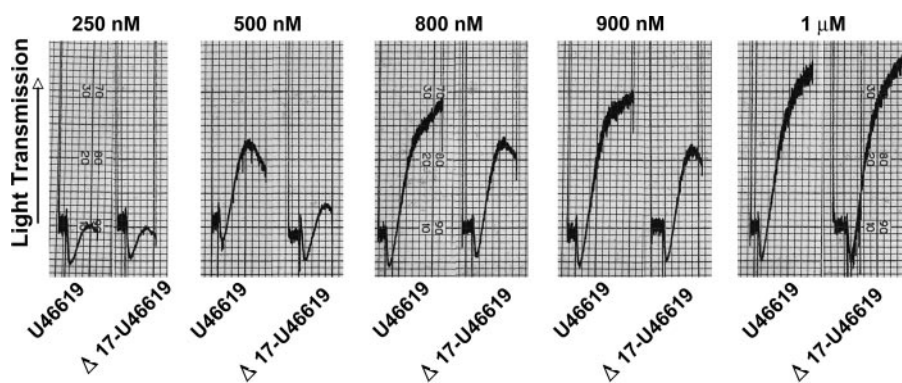
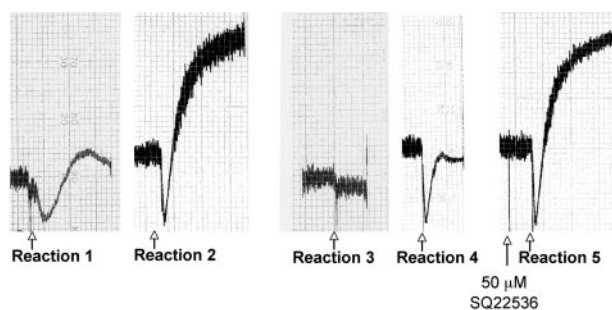


FIGURE 6. Comparison of potencies of U46619 and Δ^{17} U46619 for platelet aggregation. Human PRP (2.25×10^8 platelets per 0.4 ml) was treated with various concentrations of either U46619 or Δ^{17} U46619 as indicated, and platelet aggregation as indicated by the change in light transmission was recorded on an aggregometer. Shown is a representative result of four different donors. The concentration at which irreversible aggregation occurred for Δ^{17} U46619 was 1.2 times higher than for U46619 with platelets from each donor.



Concentrations of reactants and products (nM):

Reaction:	1	2	3	4	5
TxS:	-	+	-	+	+
AA	2.3	3	EPA	13	23
HETEs	25	34	Δ^{17} HETEs	95	145
HHTe	20	199	HHTe	15	105
PGH ₂	209	11	PGH ₃	135	31
TxA ₂	0	78	TxA ₃	0	45

FIGURE 7. Platelet aggregatory properties of AA- versus EPA-derived COX-2 and TXAS products. Enzyme reactions were initiated by adding 5 μ M AA (Reactions 1 and 2) or EPA (Reactions 3, 4, and 5) to a reaction mixture containing 750 units of purified His₆-tagged huPGHS-2. A microsomal huTXAS preparation (540 mg of protein) (Reactions 2, 4, and 5) or microsomal buffer (Reactions 1 and 3) was added and the sample vortexed for an additional 10 s. An aliquot (30 μ l) of each reaction mixture was immediately added to PRP and platelet aggregation was measured. The adenylate cyclase inhibitor SQ22536 was added to the PRP 1 min prior to addition of the reaction mixture for Reaction 5. To calculate the concentrations of products from the COX-2/TXAS reactions that were added to PRP, [1-¹⁴C]AA, or [1-¹⁴C]EPA was used in place of the unlabeled substrate. The radiolabeled products were separated by TLC and the bands corresponding to fatty acid, HETE, (12-hydroxyheptadecatrienoic acid), HHT, PGH, and TxB were scraped and quantified by liquid scintillation counting and the final concentration of each product (nM) in the PRP was calculated. The concentration of TxA₂ or TxA₃ was corrected for degradation to TxB₂ or TxB₃ during the 10 s incubation as described under "Experimental Procedures."

expected to occur only to the extent that AA levels in phospholipids were decreased by ω 3 fatty acids supplanting AA.

PGD, PGE, PGF, PGI, and TxA Synthases—There are seven different synthases reportedly involved in the conversion of PG endoperoxides to what are considered to be the biologically active PGs. Including the results from the present studies, there are now data on the specificities toward PGH₂ versus PGH₃ for H-PGDS, L-PGDS, mPGES-1, PGIS, and TXAS (Fig. 9 and Table 2). Still lacking is information on cPGES (101) and mPGES-2 (101, 102) and various putative NADPH-dependent and GSH-dependent PGFSs (103, 104).

PGDSs and mPGES-1 are about one third as active with PGH₃ as PGH₂. mPGES-1 does play a role in inflammation (67, 96, 97, 105, 106), and in principle, elevated levels of EPA could increase PGH₃ production and decrease PGH₂ formation, and the net effect would be decreased formation of PGE₂ with less than a corresponding increase in PGE₃. However, PGHS-2 appears to be the most relevant enzyme in inflammation and, as discussed above, increases in EPA have a relatively modest effect on the formation of PGH₂. PGE formation can occur via PGHS-1 and mPGES-1

(107). There are functions such as salt and water metabolism in the kidney that involve these two enzymes, and renal PGE₂ synthesis is diminished with no detectable production of PGE₃ in rats fed diets having elevated levels of fish oil (108).

PGIS and TXAS are reported to be similarly reactive with PGH₂ and PGH₃ (45, 71). This suggests that any effects of changes in tissue EPA/AA levels on PGI and TxA formation would occur primarily at the level of PGHSs and not PGIS or TXAS.

Prostanoid Receptors—There are nine G-protein-linked PG receptors. Previous comparisons of receptor specificities for the 2- versus 3-series PGs had been performed for the DP1 (70), DP2 (110, 111), EP1 (54), TP (69), and IP (69, 112) receptors. In all cases except for the TP receptor, there was little or no difference in the potencies of the 2- versus 3-series PGs. We performed both binding measurements and measurements of receptor potencies for all of the human receptors except the IP and DP receptors. In the case of the IP receptor, we analyzed potencies of purified PGI₂ versus PGI₃ using human platelets.

The EP1, EP2, and EP3 receptors bound less well and were less responsive to PGE₃ than PGE₂. As recently reviewed by Narumiya and coworkers (113), each of these receptor subtypes participate in a large number of functions each of which has the potential to be affected by increased tissue EPA/AA levels. However again, it should be noted that functions most likely to be affected are those that would be mediated via PGHS-1 and mPGES-1.

The FP receptor is known to be involved in parturition. Mice lacking cPLA₂ (114, 115), PGHS-1 (88, 89), or the FP receptor (116) have failures of parturition. Interestingly, this is also a characteristic of essential fatty acid deficiency that can be overcome with omega-6 but not omega-3 fatty acids (117–119). This could be accounted for by the low activity of PGHS-1 with EPA and the low potency of PGF_{3 α} with the FP receptor. It is not clear what enzyme is responsible for PGF_{2 α} formation *in vivo*, so we did not examine the PGF synthases that have been described (103) for their specificities toward PGH₂ versus PGH₃.

An unexpected observation in our studies of PG receptors was that TxA₃ is almost as active as TxA₂ with the TP receptor. Earlier studies indicating that TxA₃ is inactive in platelet aggre-

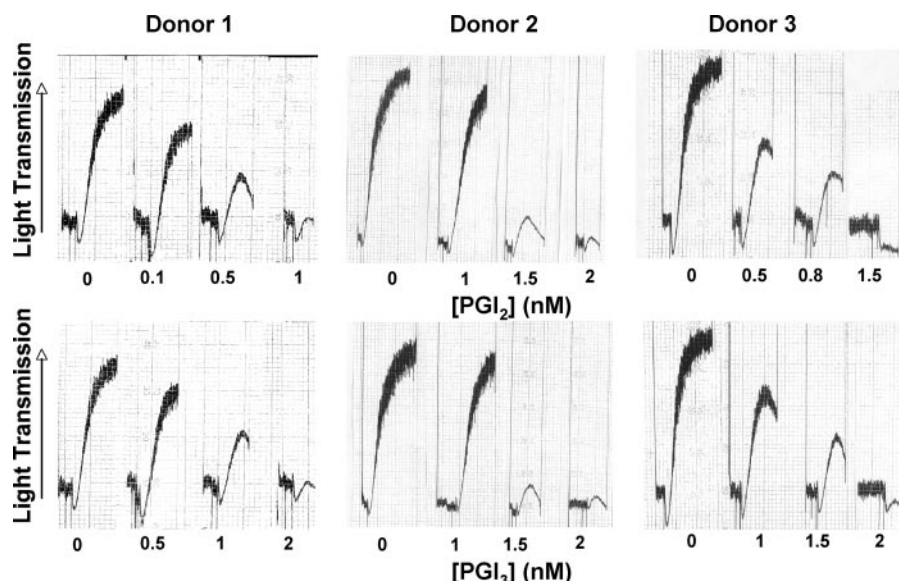


FIGURE 8. **Comparison of anti-aggregatory potencies of PGI₂ and PGI₃.** Human PRP from three donors was treated with the indicated concentrations of either PGI₂ or PGI₃ for 10 s, followed by treatment with 2 μ M U46619 to induce aggregation. Aggregation was measured as an increase in light transmission using a platelet aggregometer. Upper panels are PGI₂, and lower panels are PGI₃. Agonist was introduced at points indicated by the arrows.

dialdehyde whereas spontaneous conversion of PGH₂ to the homologous products is relatively slow. This is apparent in Fig. 3 where there is an accumulation of HHTE but not HHTe.

To the extent that we have discussed our biochemical data in the context of the biological changes seen with dietary fish oil, we have assumed simple linear relationships based on K_m , V_{max} , and EC_{50} values for the various enzymes and receptors. All of these values were obtained under optimal *in vitro* conditions. Obviously, what occurs *in vivo* cannot yet be predicted with any certainty because the ratios of enzymes and receptors to substrates and agonists involved in PG signaling may well be different *in vivo* (89). There may also be other eicosanoid mediators, including those derived from

omega-3 fatty acids that are importantly involved in PG signaling (34, 35).

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REFERENCES

1. Lands, W., Libelt, B., Morris, A., Kramer, N., Prewitt, T., Bowen, P., Schmeisser, D., Davidson, M., and Burns, J. (1992) *Biochim. Biophys. Acta* **1180**, 147–162
2. Marszalek, J., and Lodish, H. (2005) *Annu. Rev. Cell Dev. Biol.* **21**, 633–657
3. Anti, M., Marra, G., Armelao, F., Bartoli, G. M., Ficarelli, R., Percesepe, A., De Vitis, I., Maria, G., Sofo, L., and Rapaccini, G. L. (1992) *Gastroenterology* **103**, 883–891
4. Huang, Y. C., Jessup, J. M., Forse, R. A., Flickner, S., Pleskow, D., Anastopoulos, H. T., Ritter, V., and Blackburn, G. L. (1996) *Lipids* **31**, (suppl.) S313–S317
5. Kromhout, D., Bosschieter, E. B., and Coulander, C. (1985) *N. Engl. J. Med.* **312**, 1205–1209
6. Dolecek, T., and Granditis, G. (1991) *World Rev. Nutr. Diet.* **66**, 205–216
7. Kromhout, D., Feskens, E., and Bowles, C. (1995) *Int. J. Epidemiol.* **24**, 340–345
8. Daviglus, M. L., Stamler, J., Orenca, A. J., Dyer, A. R., Liu, K., Greenland, P., Walsh, M. K., Morris, D., and Shekelle, R. B. (1997) *N. Engl. J. Med.* **336**, 1046–1053
9. Zhang, J., Sasaki, S., Amano, K., and Kesteloot, H. (1999) *Prev. Med.* **28**, 520–529
10. De Caterina, R., and Zampolli, A. (2001) *Lipids* **36**, (suppl.) S69–S78
11. Hu, F. B., Bronner, L., Willett, W. C., Stampfer, M. J., Rexrode, K. M., Albert, C. M., Hunter, D., and Manson, J. E. (2002) *J. Am. Med. Assoc.* **287**, 1815–1821
12. Leaf, A., Kang, J. X., Xiao, Y.-F., and Billman, G. E. (2003) *Circulation* **107**, 2646–2652

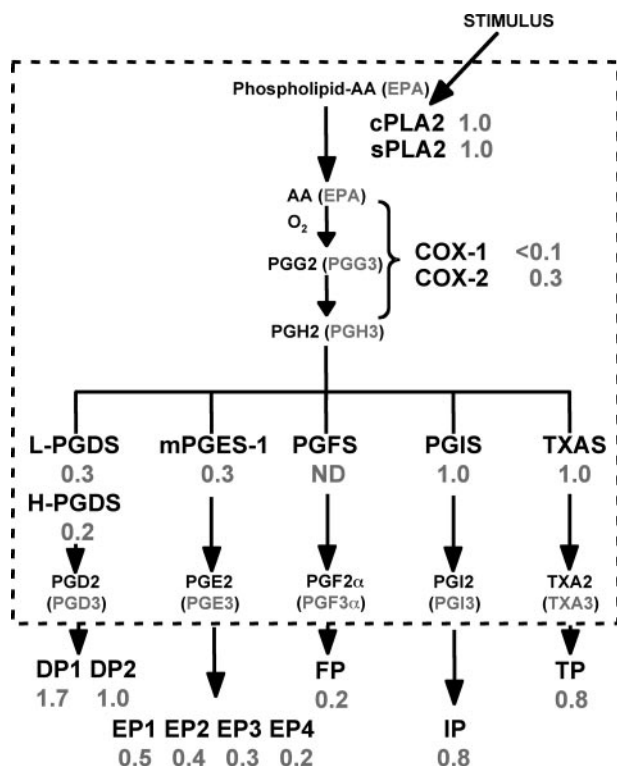


FIGURE 9. **Effectiveness of AA-derived versus EPA-derived substrates and products with enzymes and receptors of the PG pathway.** Abbreviations: TXAS, thromboxane A synthase; DP, EP, FP, IP, and TP are receptors for PGD, PGE, PGF, PGI, and TxA/PGH, respectively. ND, not determined.

gation (69) were probably compromised by the formation of PGD₃ from PGH₃ and PGD₃ being a potent anti-aggregatory compound (70). In general, PGH₃ appears to be significantly less stable than PGH₂ in the aqueous systems used for our enzyme assays; PGH₃ is rapidly converted to HHTE and malon-

13. Kris-Etherton, P. M., Harris, W. S., Appel, L. J., and for the Nutrition Committee (2003) *Arterioscler. Thromb. Vasc. Biol.* **23**, 20–30
14. Jho, D. H., Cole, S. M., Lee, E. M., and Espot, N. J. (2004) *Integr. Cancer Ther.* **3**, 98–111
15. Zeyda, M., Staffler, G., Horejsi, V., Waldhausl, W., and Stulnig, T. M. (2002) *J. Biol. Chem.* **277**, 28418–28423
16. Lee, J. Y., Plakidas, A., Lee, W. H., Heikkinen, A., Chanmugam, P., Bray, G., and Hwang, D. H. (2003) *J. Lipid Res.* **44**, 479–486
17. Gawrisch, K., Eldho, N., and Holte, L. (2003) *Lipids* **38**, 445–452
18. Fan, Y.-Y., Ly, L. H., Barhoumi, R., McMurray, D. N., and Chapkin, R. S. (2004) *J. Immunol.* **173**, 6151–6160
19. Wang, Y., Botelho, A., Martinez, G., and Brown, M. (2002) *J. Am. Chem. Soc.* **124**, 7690–7701
20. Mitchell, D., Niu, S., and Litman, B. (2003) *J. Pediatr.* **143**, (4 Suppl.), S80–S86
21. Niu, S.-L., Mitchell, D. C., Lim, S.-Y., Wen, Z.-M., Kim, H.-Y., Salem, N., Jr., and Litman, B. J. (2004) *J. Biol. Chem.* **279**, 31098–31104
22. Glomset, J. A. (2006) *Sci. STKE* **2006**, pe6
23. Jump, D. (2002) *Curr. Opin. Lipidol.* **13**, 155–164
24. Jump, D. (2004) *Crit. Rev. Clin. Lab. Sci.* **41**, 41–78
25. Schachter, J., Lester, D., and Alkon, D. (1996) *Biochim. Biophys. Acta* **1291**, 167–176
26. Pessach, I., Leto, T. L., Malech, H. L., and Levy, R. (2001) *J. Biol. Chem.* **276**, 33495–33503
27. Sano, Y., Inamura, K., Miyake, A., Mochizuki, S., Kitada, C., Yokoi, H., Nozawa, K., Okada, H., Matsushime, H., and Furuichi, K. (2003) *J. Biol. Chem.* **278**, 27406–27412
28. Cao, Y., Pearman, A. T., Zimmerman, G. A., McIntyre, T. M., and Prescott, S. M. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97**, 11280–11285
29. Smith, W. L., and Murphy, R. C. (2002) in *Biochemistry of Lipids, Lipoproteins and Membranes* (Vance, D. E., and Vance, J. E., eds) 4th Ed., Elsevier Science Publishers B.V., Amsterdam
30. Smith, W. L., DeWitt, D. L., and Garavito, R. M. (2000) *Annu. Rev. Biochem.* **69**, 149–182
31. Cowart, L. A., Wei, S., Hsu, M.-H., Johnson, E. F., Krishna, M. U., Falck, J. R., and Capdevila, J. H. (2002) *J. Biol. Chem.* **277**, 35105–35112
32. Kieran, N., Maderna, P., and Godson, C. (2004) *Kidney Int.* **65**, 1145–1154
33. Serhan, C. N., Gotlinger, K., Hong, S., Lu, Y., Siegelman, J., Baer, T., Yang, R., Colgan, S. P., and Petasis, N. A. (2006) *J. Immunol.* **176**, 1848–1859
34. Serhan, C. N., Brain, S. D., Buckley, C. D., Gilroy, D. W., Haslett, C., O'Neill, L. A. J., Perretti, M., Rossi, A. G., and Wallace, J. L. (2007) *FASEB J.* **21**, 325–332
35. Gao, L., Yin, H., Milne, G. L., Porter, N. A., and Morrow, J. D. (2006) *J. Biol. Chem.* **281**, 14092–14099
36. Braden, G., Knapp, H., Fitzgerald, D., and FitzGerald, G. (1990) *Circulation* **82**, 178–187
37. Bortuzzo, C., Hanif, R., Kashfi, K., Staiano-Coico, L., Shiff, S., and Rigas, B. (1996) *Biochim. Biophys. Acta* **1300**, 240–246
38. Petrik, M. B. H., McEntee, M. F., Chiu, C.-H., and Whelan, J. (2000) *J. Nutr.* **130**, 1153–1158
39. Song, I., Ball, T. M., and Smith, W. L. (2001) *Biochem. Biophys. Res. Commun.* **289**, 869–875
40. Yuan, C., Rieke, C. J., Rimon, G., Wingerd, B. A., and Smith, W. L. (2006) *Proc. Natl. Acad. Sci. U. S. A.* **103**, 6142–6147
41. Mbonye, U. R., Wada, M., Rieke, C. J., Tang, H.-Y., DeWitt, D. L., and Smith, W. L. (2006) *J. Biol. Chem.* **281**, 35770–35778
42. Urade, Y., Fujimoto, N., Ujihara, M., and Hayaishi, O. (1987) *J. Biol. Chem.* **262**, 3820–3825
43. Tanaka, Y., Ward, S., and Smith, W. (1987) *J. Biol. Chem.* **262**, 1374–1381
44. Yokoyama, C., Miyata, A., Suzuki, K., Nishikawa, Y., Yoshimoto, T., Yamamoto, S., Nusing, R., Ullrich, V., and Tanabe, T. (1993) *FEBS Lett.* **318**, 91–94
45. Wada, M., Yokoyama, C., Hatae, T., Shimonishi, M., Nakamura, M., Imai, Y., Ullrich, V., and Tanabe, T. (2004) *J. Biochem. (Tokyo)* **135**, 455–463
46. Kotani, M., Tanaka, I., Ogawa, Y., Usui, T., Tamura, N., Mori, K., Narumiya, S., Yoshimi, T., and Nakao, K. (1997) *Genomics* **40**, 425–434
47. Regan, J. (2003) *Life Sci.* **74**, 143–153
48. Fujino, H., and Regan, J. W. (2006) *Mol. Pharmacol.* **69**, 5–10
49. Fujino, H., Srinivasan, D., and Regan, J. W. (2002) *J. Biol. Chem.* **277**, 48786–48795
50. Wilson, S. J., and Smyth, E. M. (2006) *J. Biol. Chem.* **281**, 11780–11786
51. Zhang, L., DiLizio, C., Kim, D., Smyth, E. M., and Manning, D. R. (2006) *Mol. Pharmacol.* **69**, 1433–1440
52. Pratico, D., Smyth, E. M., Violi, F., and FitzGerald, G. A. (1996) *J. Biol. Chem.* **271**, 14916–14924
53. Funk, C. D., Furci, L., FitzGerald, G. A., Grygorczyk, R., Rochette, C., Bayne, M. A., Abramovitz, M., Adam, M., and Metters, K. M. (1993) *J. Biol. Chem.* **268**, 26767–26772
54. Ungrin, M. D., Carriere, M.-C., Denis, D., Lamontagne, S., Sawyer, N., Stocco, R., Tremblay, N., Metters, K. M., and Abramovitz, M. (2001) *Mol. Pharmacol.* **59**, 1446–1456
55. Fujino, H., Pierce, K. L., Srinivasan, D., Protzman, C. E., Krauss, A. H., Woodward, D. F., and Regan, J. W. (2000) *J. Biol. Chem.* **275**, 29907–29914
56. Fisher, S. K., Domask, L. M., and Roland, R. M. (1989) *Mol. Pharmacol.* **35**, 195–204
57. Liu, W., Cao, D., Oh, S. F., Serhan, C. N., and Kulmacz, R. J. (2006) *FASEB J.* **20**, 1097–1108
58. Kulmacz, R. J., Pendleton, R. B., and Lands, W. E. M. (1994) *J. Biol. Chem.* **269**, 5527–5536
59. Laneuville, O., Breuer, D. K., Xu, N., Huang, Z. H., Gage, D. A., Watson, J. T., Lagarde, M., DeWitt, D. L., and Smith, W. L. (1995) *J. Biol. Chem.* **270**, 19330–19336
60. Morita, I., Takahashi, R., Saito, Y., and Murota, S. (1983) *J. Biol. Chem.* **258**, 10197–10199
61. Lands, W. E. M., LeTellier, P. R., Rome, L. H., and Vanderhoek, J. Y. (1973) *Adv. Biosci.* **9**, 15–28
62. Kulmacz, R. (2005) *Biochem. Biophys. Res. Commun.* **338**, 25–33
63. Pinzar, E., Miyano, M., Kanaoka, Y., Urade, Y., and Hayaishi, O. (2000) *J. Biol. Chem.* **275**, 31239–31244
64. Kanaoka, Y., and Urade, Y. (2003) *Prostaglandins Leukot. Essent. Fatty Acids* **69**, 163–167
65. Urade, Y., Tanaka, T., Eguchi, N., Kikuchi, M., Kimura, H., Toh, H., and Hayaishi, O. (1995) *J. Biol. Chem.* **270**, 1422–1428
66. Urade, Y., Fujimoto, N., and Hayaishi, O. (1985) *J. Biol. Chem.* **260**, 12410–12415
67. Thoren, S., Weinander, R., Saha, S., Jegerschold, C., Pettersson, P. L., Samuelsson, B., Hebert, H., Hamberg, M., Morgenstern, R., and Jakobsen, P.-J. (2003) *J. Biol. Chem.* **278**, 22199–22209
68. Ouellet, M., Falgout, J. P., Ear, P. H., Pen, A., Mancini, J. A., Riendeau, D., and Percival, M. D. (2002) *Protein Expression Purif.* **26**, 489–495
69. Needleman, P., Raz, A., Minkes, M. S., Ferrendelli, J. A., and Sprecher, H. (1979) *Proc. Natl. Acad. Sci. U. S. A.* **76**, 944–948
70. Whitaker, M. O., Wyche, A., Fitzpatrick, F., Sprecher, H., and Needleman, P. (1979) *Proc. Natl. Acad. Sci. U. S. A.* **76**, 5919–5923
71. Hecker, M., and Ullrich, V. (1989) *J. Biol. Chem.* **264**, 141–150
72. Ghosh, M., Loper, R., Ghomashchi, F., Tucker, D. E., Bonventre, J. V., Gelb, M. H., and Leslie, C. C. (2007) *J. Biol. Chem.* **282**, 11676–11686
73. Hanel, A., Schuttel, S., and Gelb, M. (1993) *Biochemistry* **32**, 5949–5958
74. Mahadevappa, V., and Holub, B. (1987) *J. Lipid Res.* **28**, 1275–1280
75. Clark, J., Schievella, A., Nalefski, E., and Lin, L. (1995) *J. Lipid. Mediat. Cell Signal.* **12**, 83–117
76. Kramer, R., and Sharp, J. (1997) *FEBS Lett.* **410**, 49–53
77. Shikano, M., Masuzawa, Y., Yazawa, K., Takayama, K., Kudo, I., and Inoue, K. (1994) *Biochim. Biophys. Acta* **1212**, 211–216
78. Strokim, M., Sergeeva, M., and Reiser, G. (2003) *Br. J. Pharmacol.* **139**, 1014–1022
79. Satake, Y., Diaz, B. L., Balestrieri, B., Lam, B. K., Kanaoka, Y., Grusby, M. J., and Arm, J. P. (2004) *J. Biol. Chem.* **279**, 16488–16494
80. Mounier, C. M., Ghomashchi, F., Lindsay, M. R., James, S., Singer, A. G., Parton, R. G., and Gelb, M. H. (2004) *J. Biol. Chem.* **279**, 25024–25038
81. Dennis, E. A. (1994) *J. Biol. Chem.* **269**, 13057–13060
82. Capdevila, J. H., Morrow, J. D., Belosludtsev, Y. Y., Beauchamp, D. R.,

- DuBois, R. N., and Falck, J. R. (1995) *Biochemistry* **34**, 3325–3337
83. Kulmacz, R. J., and Wang, L. H. (1995) *J. Biol. Chem.* **270**, 24019–24023
 84. Aronoff, D. M., Oates, J. A., and Boutaud, O. (2006) *Clin. Pharmacol. Ther.* **79**, 9–19
 85. Malkowski, M. G., Ginell, S., Smith, W. L., and Garavito, R. M. (2000) *Science* **289**, 1933–1937
 86. Thuresson, E. D., Lakkides, K. M., Rieke, C. J., Sun, Y., Wingerd, B. A., Micielli, R., Mulichak, A. M., Malkowski, M. G., Garavito, R. M., and Smith, W. L. (2001) *J. Biol. Chem.* **276**, 10347–10357
 87. Malkowski, M. G., Thuresson, E. D., Lakkides, K. M., Rieke, C. J., Micielli, R., Smith, W. L., and Garavito, R. M. (2001) *J. Biol. Chem.* **276**, 37547–37555
 88. Langenbach, R., Morham, S. G., Tian, H. F., Loftin, C. D., Ghanayem, B. I., Chulada, P. C., Mahler, J. F., Lee, C. A., Goulding, E. H., Kluckman, K. D., Kim, H. S., and Smithies, O. (1995) *Cell* **83**, 483–492
 89. Yu, Y., Cheng, Y., Fan, J., Chen, X.-S., Klein-Szanto, A., FitzGerald, G. A., and Funk, C. D. (2005) *J. Clin. Invest.* **115**, 986–995
 90. Kanaoka, Y., Maekawa, A., Penrose, J. F., Austen, K. F., and Lam, B. K. (2001) *J. Biol. Chem.* **276**, 22608–22613
 91. Goulet, J. L., Pace, A. J., Key, M. L., Byrum, R. S., Nguyen, M., Tilley, S. L., Morham, S. G., Langenbach, R., Stock, J. L., McNeish, J. D., Smithies, O., Coffman, T. M., and Koller, B. H. (2004) *J. Immunol.* **173**, 1321–1326
 92. Reddy, S. T., and Herschman, H. R. (1994) *J. Biol. Chem.* **269**, 15473–15480
 93. Smith, W. L., and Langenbach, R. (2001) *J. Clin. Invest.* **107**, 1491–1495
 94. Yu, Y., Fan, J., Hui, Y., Rouzer, C. A., Marnett, L. J., Klein-Szanto, A. J., FitzGerald, G. A., and Funk, C. D. (2007) *J. Biol. Chem.* **282**, 1498–1506
 95. Ni, Z., Okeley, N. M., Smart, B. P., and Gelb, M. H. (2006) *J. Biol. Chem.* **281**, 16245–16255
 96. Boulet, L., Ouellet, M., Bateman, K. P., Ethier, D., Percival, M. D., Riendeau, D., Mancini, J. A., and Methot, N. (2004) *J. Biol. Chem.* **279**, 23229–23237
 97. Kamei, D., Yamakawa, K., Takegoshi, Y., Mikami-Nakanishi, M., Nakatani, Y., Oh-ishi, S., Yasui, H., Azuma, Y., Hirasawa, N., Ohuchi, K., Kawaguchi, H., Ishikawa, Y., Ishii, T., Uematsu, S., Akira, S., Murakami, M., and Kudo, I. (2004) *J. Biol. Chem.* **279**, 33684–33695
 98. Peters-Golden, M., Canetti, C., Mancuso, P., and Coffey, M. J. (2005) *J. Immunol.* **174**, 589–594
 99. Masferrer, J. L., Zweifel, B. S., Manning, P. T., Hauser, S. D., Leahy, K. M., Smith, W. G., Isakson, P. C., and Seibert, K. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91**, 3228–3232
 100. Seibert, K., Zhang, Y., Leahy, K., Hauser, S., Masferrer, J., Perkins, W., Lee, L., and Isakson, P. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91**, 12013–12017
 101. Murakami, M., and Kudo, I. (2004) *Prog. Lipid Res.* **43**, 3–35
 102. Murakami, M., Nakashima, K., Kamei, D., Masuda, S., Ishikawa, Y., Ishii, T., Ohmiya, Y., Watanabe, K., and Kudo, I. (2003) *J. Biol. Chem.* **278**, 37937–37947
 103. Watanabe, K. (2002) *Prostaglandins Other Lipid Mediat.* **68–69**, 401–407
 104. Wacławik, A., Rivero-Muller, A., Blitek, A., Kaczmarski, M. M., Brokken, L. J. S., Watanabe, K., Rahman, N. A., and Ziecik, A. J. (2006) *Endocrinology* **147**, 210–221
 105. Murakami, M., and Kudo, I. (2006) *Curr. Pharm. Des.* **12**, 943–954
 106. Cheng, Y., Wang, M., Yu, Y., Lawson, J., Funk, C. D., and FitzGerald, G. A. (2006) *J. Clin. Invest.* **116**, 1391–1399
 107. Schneider, A., Zhang, Y., Zhang, M., Lu, W., Rao, R., Fan, X., Redha, R., Davis, L., Breyer, R., Harris, R., Guan, Y., and Breyer, M. (2004) *Kidney Int.* **65**, 1205–1213
 108. Hansen, H., and Jensen, B. (1983) *Lipids* **18**, 682–690
 109. Murakami, M., Nakatani, Y., Tanioka, T., and Kudo, I. (2002) *Prostaglandins Other Lipid Mediat.* **68–69**, 383–399
 110. Hirai, H., Tanaka, K., Yoshie, O., Ogawa, K., Kenmotsu, K., Takamori, Y., Ichimasa, M., Sugamura, K., Nakamura, M., Takano, S., and Nagata, K. (2001) *J. Exp. Med.* **193**, 255–262
 111. Monneret, G., Cossette, C., Gravel, S., Rokach, J., and Powell, W. S. (2003) *J. Pharmacol. Exp. Ther.* **304**, 349–355
 112. Kobzar, G., Mardla, V., Jarving, I., and Samel, N. (2001) *Cell Physiol. Biochem.* **11**, 279–284
 113. Sugimoto, Y., and Narumiya, S. (2007) *J. Biol. Chem.* **282**, 11613–11617
 114. Uozumi, N., Kume, K., Nagase, T., Nakatani, N., Ishii, S., Tashiro, F., Komagata, Y., Maki, K., Ikuta, K., Ouchi, Y., Miyazaki, J., and Shimizu, T. (1997) *Nature* **390**, 618–622
 115. Bonventre, J., Huang, Z., Taheri, M., O'Leary, E., Li, E., Moskowitz, M., and Saperstein, A. (1997) *Nature* **390**, 622–625
 116. Sugimoto, Y., Yamasaki, A., Segi, E., Tsuboi, K., Aze, Y., Nishimura, T., Oida, H., Yoshida, N., Tanaka, T., Katsuyama, M., Hasumoto, K., Murata, T., Hirata, M., Ushikubi, F., Negishi, M., Ichikawa, A., and Narumiya, S. (1997) *Science* **277**, 681–683
 117. Burr, G. O., Burr, M. M., and Miller, E. S. (1932) *J. Biol. Chem.* **97**, 1–9
 118. Quackenbush, F. W., Kummerow, F. A., and Steenbock, H. (1942) *J. Nutr.* **24**, 213–224
 119. Mohrhauer, H., and Holman, R. T. (1963) *J. Lipid Res.* **4**, 151–159
 120. Tanikawa, N., Ohmiya, Y., Ohkubo, H., Hashimoto, K., Kangawa, K., Kojima, M., Ito, S., and Watanabe, K. (2002) *Biochem. Biophys. Res. Commun.* **291**, 884–889

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Masayuki Wada, Cynthia J. DeLong, Yu H. Hong, Caroline J. Rieke, Inseok Song,
Ranjinder S. Sidhu, Chong Yuan, Mark Warnock, Alvin H. Schmaier, Chieko
Yokoyama, Emer M. Smyth, Stephen J. Wilson, Garret A. FitzGerald, R. Michael
Garavito, De Xin Sui, John W. Regan and William L. Smith

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