Proc. Natl. Acad. Sci. USA
Vol. 76, No. 2, pp. 944-948, February 1979
Medical Sciences

Triene prostaglandins: Prostacyclin and thromboxane biosynthesis and unique biological properties

(5,8,11,14,17-eicosapentaenoic acid/endoperoxides)

PHILIP NEEDLEMAN*, AMIRAM RAZ*, MARK S. MINKES*, JAMES A. FERRENDELLI*, AND HOWARD SPRECHER†

*Departments of Pharmacology and Surgery, Washington University School of Medicine, St. Louis, Missouri 63110; and †Department of Physiological Chemistry, Ohio State University, Columbus, Ohio 43210

Communicated by Oliver H. Lowry, November 6, 1978

ABSTRACT **Platelets enzymatically convert prostaglandin H_3 (PGH_3) into thromboxane A_3. Both PGH_2 and thromboxane A_2 aggregate human platelet-rich plasma. In contrast, PGH_3 and thromboxane A_3 do not. PGH_3 and thromboxane A_3 increase platelet cyclic AMP in platelet-rich plasma and thereby: (*i*) inhibit aggregation by other agonists, (*ii*) block the ADP-induced release reaction, and (*iii*) suppress platelet phospholipase-A_2 activity or events leading to its activation. PGI_3 (Δ^{17}-prostacyclin; synthesized from PGH_3 by blood vessel enzyme) and PGI_2 (prostacyclin) exert similar effects. Both compounds are potent coronary relaxants that also inhibit aggregation in human platelet-rich plasma and increase platelet adenylate cyclase activity. Radioactive eicosapentaenoate and arachidonate are readily and comparably acylated into platelet phospholipids. In addition, stimulation of prelabeled platelets with thrombin releases comparable amounts of eicosapentaenoate and arachidonate, respectively. Although eicosapentaenoic acid is a relatively poor substrate for platelet cyclooxygenase, it appears to have a high binding affinity and thereby inhibits arachidonic acid conversion by platelet cyclooxygenase and lipoxygenase. It is therefore possible that the triene prostaglandins are potential antithrombotic agents because their precursor fatty acids, as well as their transformation products, PGH_3, thromboxane A_3, and PGI_3, are capable of interfering with aggregation of platelets in platelet-rich plasma.**

We previously demonstrated (1) that the fatty acid 5,8,11,14,17-eicosapentaenoic acid (C20:5) was converted by sheep seminal vesicle cyclooxygenase into a labile contractile substance that was a mixture of prostaglandin (PG) endoperoxides PGG_3 and PGH_3 (1). The 3-series endoperoxides were then enzymatically converted by platelet microsomes into a potent labile vasoconstrictor that was presumed to be thromboxane A_3 (1, 2). In addition, application of purified PGH_2 (produced from arachidonic acid) or PGH_3 to isolated spiral strips of bovine coronary artery caused a transient relaxation, whereas PGH_1 (produced by 8,11,14-eicosatrienoic acid) contracted the coronary strip (3). The primary product generated by bovine coronary arteries or by isolated perfused rabbit hearts from [^{14}C]arachidonic acid was 6-keto-$PGF_{1\alpha}$ (4–6), the stable end product formed from prostacyclin (PGI_2) (7) whereas [^{14}C]eicosatrienoic acid was only converted to PGE_1 (4, 8). These results indicated that the Δ^5 double bond of PG endoperoxides is required for prostacyclin synthesis. Thus, PGH_2 is the precursor of PGI_2 and its stable aqueous end product 6-keto-$PGF_{1\alpha}$, and PGH_3 which is an active coronary relaxant was presumably converted to PGI_3 and ultimately degraded to its presumed end product Δ^{17}-6-keto-$PGF_{1\alpha}$. However, no direct chemical or biological proof of this latter pathway has been reported.

Comparative study of the actions of metabolites of arachidonate and eicosapentaenoate on platelets in platelet-rich plasma (PRP) produced unexpected results. Arachidonate, PGH_2, and thromboxane A_2 were potent aggregators of human platelets but, in sharp contrast, the eicosapentaenoic acid, PGH_3, or thromboxane A_3 did not cause platelet aggregation (1, 2). Recently, this latter observation was given potential physiological perspective by the finding that Eskimos who have a bleeding tendency have elevated eicosapentaenoate and depressed arachidonate levels in their blood lipid fraction (9). It was suggested that endogenous PGI_3 synthesis from eicosapentaenoate by vasculature contributed to the bleeding tendency (9). However, no direct evidence of PGI_3 synthesis was presented, such as isolation and chromatographic identification of products or abolition of the synthesis of the antithrombotic substance with a prostacyclin synthetase inhibitor such as 15-hydroperoxyarachidonic acid. In addition, there is no evidence to indicate if the Eskimos' bleeding disorder is due to a coagulation defect or to a platelet defect.

In the current investigation we document, biologically and chemically, the synthesis of PGI_3 and its inactive metabolite Δ^{17}-6-keto-$PGF_{1\alpha}$ and of thromboxane A_3 and its metabolite thromboxane B_3. Furthermore, we analyze the unique actions of the triene PGs on human PRP and demonstrate an intrinsic platelet mechanism whereby PGH_3 or thromboxane A_3 inhibits aggregation by pro-aggregatory molecules.

MATERIALS AND METHODS

Materials. 5,8,11,14,17-[1-^{14}C]Eicosapentaenoic acid (20:5), 7.2 Ci/mol, was prepared by total organic synthesis (10). [^{14}C]PGH_3 was enzymatically synthesized (7-min incubation) and purified as described (1, 11). 15-Hydroperoxyarachidonic acid, an *in vitro* prostacyclin synthetase inhibitor (12), was synthesized with soybean lipoxygenase (13). Bovine aorta microsomes (BAM) were used as the source of prostacyclin synthetase and were prepared from freshly dissected vessels as described (5). Aspirin-treated platelet microsomes (APM) were employed as the source of thromboxane synthetase and prepared as previously described (1).

Vascular Smooth Muscle Bioassay. The bovine coronary artery and rabbit thoracic aorta assay tissues were hung in a vertical superfusion cascade and continuously bathed with Krebs–Henseleit solution (95% O_2/5% CO_2) at 10 ml/min as described (14–16).

Platelet Aggregation. Citrated human PRP or washed platelets were prepared as described (17).

Prostacyclin and Thromboxane Generation. The endoperoxide (PGH_2 or PGH_3) in acetone was dried in a stream of N_2 and resuspended in 25 μl of phosphate buffer (50 mM, pH

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: PG, prostaglandin; PRP, platelet-rich plasma; BAM, bovine aorta microsomes; APM, aspirin-treated platelet microsomes.

7.4). Twenty microliters (80 μg of protein) of BAM was added to the endoperoxides and incubated for 1 min at room temperature.

Twenty-five microliters (200 μg of protein) of APM was added to endoperoxide dissolved in phosphate buffer and incubated at 0°C for 2 min. The thromboxane synthetase inhibitor imidazole (18) was preincubated (5 mM) with the APM (or intact platelets) for 5 min.

Platelet Radiochemical Experiments. The ^{14}C-labeled endoperoxide ([^{14}C]PGH_2 at 100,000 cpm or [^{14}C]PGH_3 at 60,000 cpm) was incubated with washed platelets (0.4 ml) at 37°C for 15 min, acidified to pH 3.5 with 2M formic acid, and extracted twice with 2 vol of ethyl acetate. The extract was dried and applied together with unlabeled PG standards (kindly supplied by The Upjohn Company) to silica gel plates. The solvent system was benzene/dioxane/acetic acid, 60:30:3 (vol/vol).

[^{14}C]Prostacyclin Generation. The ^{14}C-labeled endoperoxides were dissolved in 25 μl of phosphate buffer and incubated with 25 μl of BAM at 37°C for 5 min. Acidification and extraction were carried out as described above. The solvent system (system A-9) was the organic phase from ethyl acetate/acetic acid/2,2,4-trimethylpentane/water, 110:20:50:100 (vol/vol).

Platelet Phospholipid Labeling Technique. Human blood was withdrawn into a syringe containing 1/13th vol of 77 mM EDTA and centrifuged for 10 min at 120 × *g*; the supernate was centrifuged at 4000 rpm for 6 min. The platelets were resuspended in 0.67 vol of albumin phosphate (33 mM, pH 6.5) containing 1 mg of glucose, 6.6 mg of NaCl, and 5 mg of fatty acid-poor bovine serum albumin per ml. [^{14}C]Arachidonic acid (5 μg, 1.67 × 10^6 cpm) or [^{14}C]eicosapentaenoic acid (25 μg, 1.6 × 10^6 cpm) was dissolved in 100 μl of Tris buffer (100 mM, pH 9.0) and added to the platelet suspension which was incubated for 30 min at 37°C. The labeled platelets were centrifuged at 2000 rpm for 5 min and resuspended in calcium-free Krebs–Henseleit medium (pH 7.4).

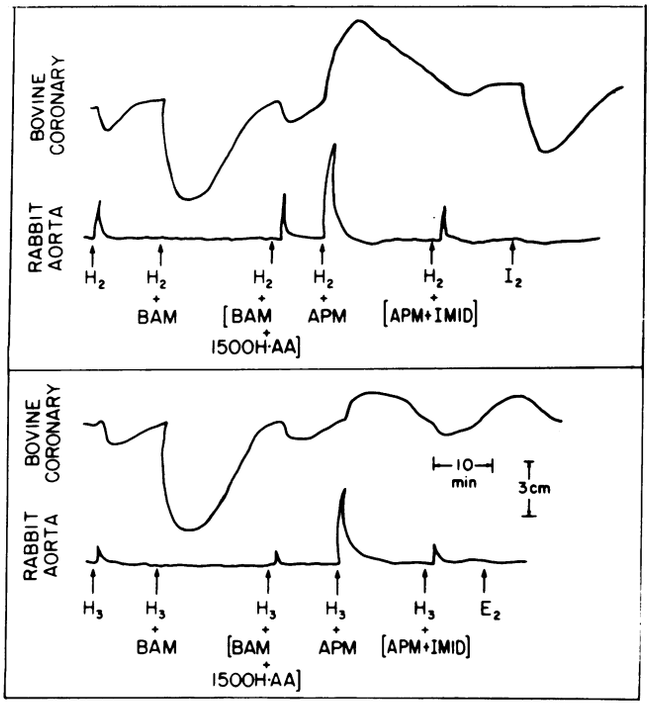

FIG. 1. Responsiveness of isolated blood vessels to various diene and triene PGs. H_2, PGH_2, 250 ng; BAM, bovine aorta microsomes, 20 μl of 4 mg of protein/ml; 15 OOH-AA, 15-hydroperoxyarachidonic acid, 3 μg; APM, aspirin-treated platelet microsomes, 25 μl of 8 mg of protein/ml; IMID, imidazole, 5 mM; I_2, PGI_2 (prostacyclin), 100 ng; H_3, PGH_3, 250 ng; E_2, PGE_2, 500 ng.

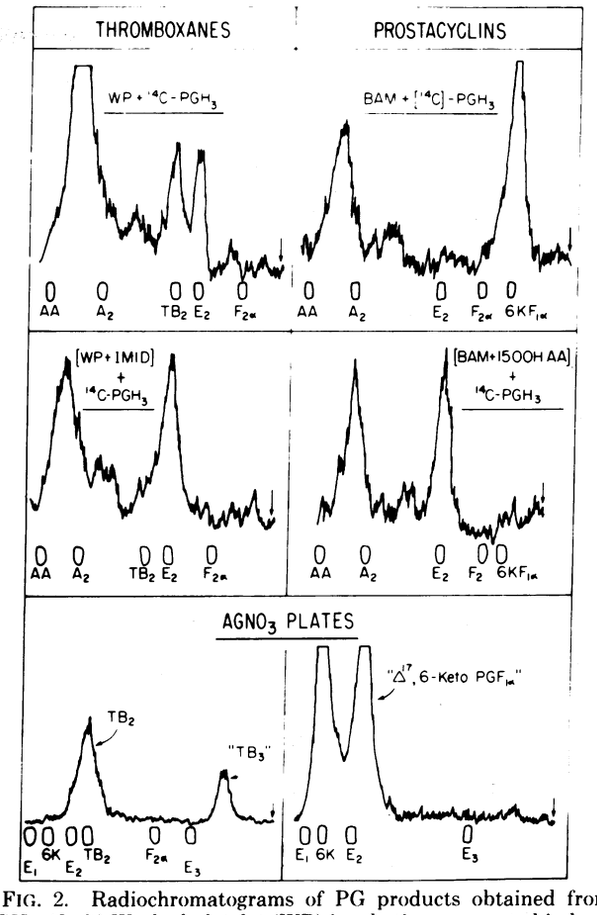

FIG. 2. Radiochromatograms of PG products obtained from PGH_3. (*Left*) Washed platelet (WP) incubations separated in benzene/dioxane/acetic acid, 60:30:3 (vol/vol). The radioactive peaks corresponding to thromboxane B_2 and thromboxane B_3 were rechromatographed on $AgNO_3$ plates in chloroform/methanol, 80:20 (vol/vol). (*Right*) Prostacyclin products. Unlabeled standards, shown at the bottom of each chromatogram, include: AA, arachidonic acid; A_2, PGA_2; TB_2, thromboxane B_2; E_2, PGE_2; $F_{2\alpha}$, $PGF_{2\alpha}$; E_1, PGE_1; E_2, PGE_2; D_2, PGD_2; $6KF_{1\alpha}$ or 6K, 6-keto-$PGF_{1\alpha}$.

Platelet Cyclic Nucleotide and ADP. Platelet cyclic AMP levels (at 60 sec) were determined by radioimmunoassay as described (19). The concentration of ADP released from aggregated platelets was measured enzymatically as described (17).

RESULTS

Biological and Radiochemical Characterization of PGI_3 and Thromboxane A_3. Application of endoperoxide PGH_2 or PGH_3 resulted in a modest coronary relaxation and an aorta contraction (Fig. 1; ref. 3). Both endoperoxides were readily converted by BAM to a powerful coronary relaxant with the concomitant loss of any intact endoperoxide as evidenced by the loss of the aorta constrictor. The enzymatically generated labile coronary relaxant is presumably PGI_3 because its synthesis is blocked by the prostacyclin synthetase inhibitor 15-hydroperoxyarachidonic acid. PGH_1, which lacks the Δ_5 double bond necessary for the prostacyclin ring structure, is not converted by BAM to a coronary relaxant (3, 4, 8). Similarly, when the endoperoxides PGH_2 and PGH_3 were incubated with platelet microsomes, potent labile constrictor substances were generated. This enzymatic generation was blocked by imidazole, a thromboxane synthetase inhibitor.

Incubation of [^{14}C]PGH_3 with washed platelets generated a radioactive peak that comigrated with thromboxane B_2 in the

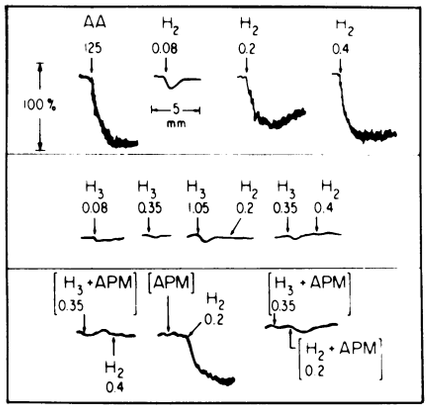

FIG. 3. Comparison of effects of diene and triene endoperoxide products (abbreviations as in Fig. 2; numbers are μg) on aggregation of human PRP.

benzene/dioxane/acetic acid solvent (Fig. 2 *left*). Pretreatment of the washed platelets with imidazole blocked the formation of a thromboxane-like peak and led to the appearance of PGE_2. The thromboxane B_2 or B_3 chromatography zone generated by the incubation of washed platelets with [^{14}C]PGH_2 or [^{14}C]-PGH_3, respectively, was extracted with chloroform/methanol, 2:1 (vol/vol) and each extract was applied to a $AgNO_3$-coated silica gel G plate; a clear separation of thromboxane B_2 from thromboxane B_3 was obtained (Fig. 2 *bottom left*). Radiochemical evidence of PGI_3 was obtained from the incubation of [^{14}C]PGH_3 with BAM which led to the formation of a product that comigrated with 6-keto-$PGF_{1\alpha}$ (Fig. 2 *right*). 15-Hydroperoxyarachidonic acid blocked formation of the triene product (Fig. 2 *middle right*). 6-Keto-$PGF_{1\alpha}$ was separable from the Δ^{17}-6-keto-$PGF_{1\alpha}$ (the presumed PGI_3 degradation product) on $AgNO_3$ plates (Fig. 2 *bottom right*).

The striking difference between the triene and diene products became apparent from studies of platelet function. Addition of arachidonate or PGH_2 (Fig. 3 *top*) or thromboxane A_2 (1) to human PRP resulted in a rapid irreversible aggregation and ADP-release reaction. In sharp contrast, neither PGH_3 nor thromboxane A_3 (i.e., PGH_3 plus APM) caused aggregation of PRP (Fig. 3 *middle*). Furthermore, pretreatment of PRP with either PGH_3 or thromboxane A_3 actually inhibited subsequent aggregation by other agonists including PGH_2 (Fig. 3 *middle*), thromboxane A_2 (Fig. 3 *bottom*), ADP, and collagen (not shown).

Effect of PGH_3 on Cyclic AMP Levels in PRP. Substances that increase platelet cyclic AMP levels inhibit platelet aggregation (20–23). Both PGH_3 (350 ng) and thromboxane A_3 increased cyclic AMP mean (±SEM) concentration (28 ± 4 and 22 ± 3 pmol of cyclic AMP per 400 μl of PRP, respectively); PGH_2 (200 ng) and thromboxane A_2 caused little or no change compared to control PRP (8 ± 5; n = 4). However, these data do not preclude the possibility that the increase in cyclic AMP induced by PGH_3 and thromboxane A_3 might be due to conversion to PGD_3 or PGE_3. PGI_3 (i.e., PGH_3 plus BAM increased cyclic AMP to 73 ± 6), PGI_2 (i.e., PGH_2 plus BAM increased cyclic AMP to 53 ± 1), and PGH_1 (300 ng increased cyclic AMP

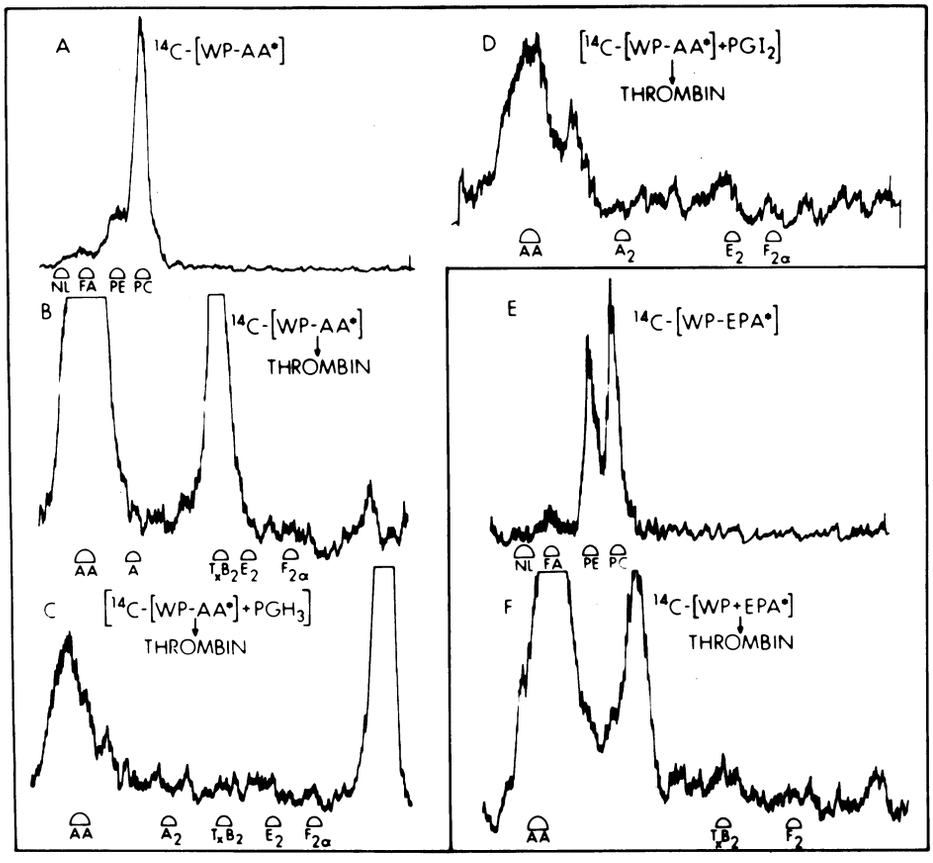

FIG. 4. Radiochromatograms obtained from prelabeled human washed platelets (WP). (*A* and *E*) Lipid extract of platelets prelabeled with [^{14}C]arachidonic acid (^{14}C-[WP-AA*]) or with [^{14}C]eicosapentaenoic acid (^{14}C-[WP-EPA*]). The labeled lipids were extracted with 20 vol of chloroform/methanol, 2:1 (vol/vol), and separated with a solvent system of chloroform/methanol/NH_4OH, 65:35:5 (vol/vol). Abbreviations: NL, neutral lipids; FA, fatty acid; PE, phosphatidylethanolamine; PC, phosphatidylcholine. (*B*, *C*, and *D*) Arachidonate-prelabeled platelets stimulated with thrombin (1 unit) in control platelets (*B*), PGH_3 pretreated (450 ng) platelets (*C*), or PGI_2-pretreated (100 ng) platelets (*D*). (*E*) Eicosapentaenoic acid-prelabeled platelets treated with thrombin.

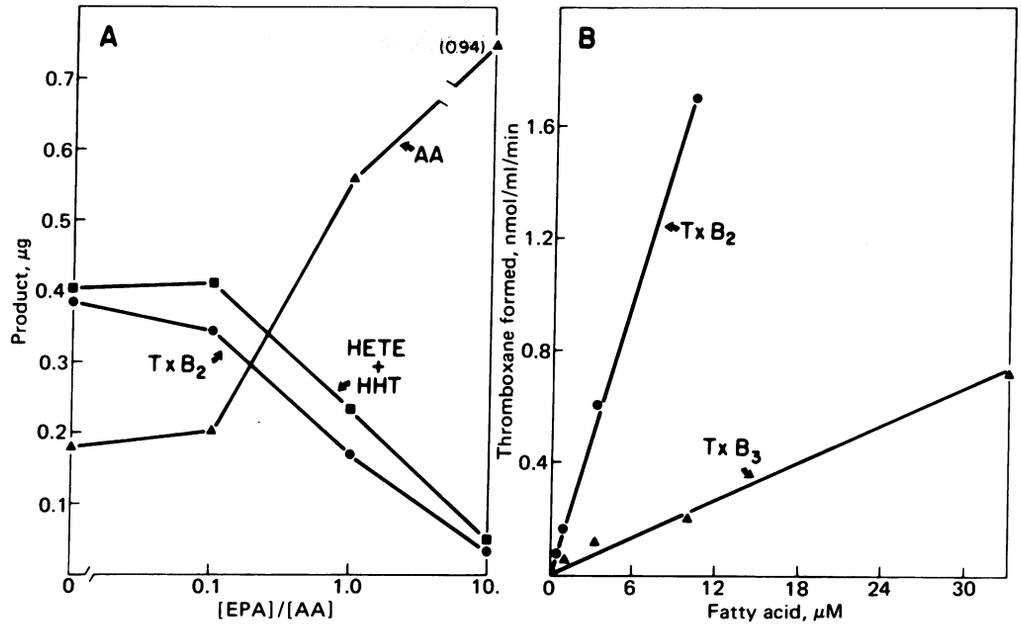

FIG. 5. Eicosapentaenoate competition with arachidonate (AA) for platelet cyclooxygenase (*A*). Conversion of [^{14}C]arachidonate (1 μg, 300,000 cpm) to products (scraped from thin-layer plates and assayed) by washed platelets (0.4 ml at 37°C mixed at 1100 rpm for 5 min) in the presence of varying amounts of unlabeled eicosapentaenoate (i.e., 0, 0.1, 1.0, and 10 μg). (*B*) Conversions of [^{14}C]arachidonate (●) incubated for 5 min with washed platelets and of [^{14}C]eicosapentaenoate (▲). HETE, 12-hydroxyeicosatetraenoic acid; HHT, hydroxyheptadecatrienoic acid; Tx, thromboxane.

to 18 ± 3) all increase platelet cyclic AMP and inhibit platelet aggregation. PGH_1 has been demonstrated to increase cyclic AMP levels in PRP (11, 23).

Effect of PGH_3 on ADP Release Reaction and Thromboxane Synthesis. Preincubation of PGH_3 with PRP prevented aggregation and suppressed the ADP release induced by exogenous arachidonic acid. Addition of 50 μg of arachidonate to PRP caused complete aggregation and release of 8 μM ADP, and high concentrations of arachidonate caused no further ADP release (data not shown). As evidence that the platelets were not lysed by the arachidonate, subsequent lysis with Triton caused aggregation. In contrast, a 50% decrease in formation of contractile activity was induced by thrombin (10 units) in PRP preincubated with PGH_3 (0.4–0.7 μM), PGI_2 (0.3–0.6 μM), or dibutyryl cyclic AMP (5 mM).

Both [^{14}C]eicosapentaenoate and [^{14}C]arachidonate were readily and similarly acylated into phospholipids when incubated with human platelets (Fig. 4 *A* and *E*). In addition, deacylation by thrombin or an ionophore (A-23187) of separately prelabeled platelets released comparable amounts of [^{14}C]-arachidonate or [^{14}C]eicosapentaenoate, respectively (Fig. 4 *B* and *F*). Pretreatment of the arachidonate-labeled platelets with PGH_3 (Fig. 4*C*), PGI_2 (Fig. 4*D*), or PGH_1 (100 ng) markedly decreased the release of fatty acid and abolished the thromboxane formation. There was no increase in fatty acid release over basal levels when thrombin was added to labeled platelets treated with PGH_3 or PGI_2 (Fig. 4 *C* and *D*).

Competition of Eicosapentaenoate with Arachidonate for Platelet Cyclooxygenase. Unlabeled eicosapentaenoic acid and [^{14}C]arachidonate were mixed in varying ratios and the reaction was initiated by the addition of washed platelets. The eicosapentaenoate effectively competed with arachidonate such that a 1:1 mixture of the fatty acids resulted in a 50% inhibition of formation of thromboxane B_2 and 12-hydroxyeicosatetraenoic and hydroxyheptadecatrienoic acids and block of arachidonic destruction (Fig. 5*A*). On the other hand, the eicosapentaenoate was a much poorer substrate for platelet cyclooxygenase, being converted only one-eighth as efficiently as arachidonate to thromboxane (Fig. 5*B*).

the release of a total of 20 μM ADP. Pretreatment of PRP with PGH_3 (100 ng) inhibited aggregation and ADP release.

Thromboxane A_2 formation was monitored directly by measuring the rabbit aorta contractile activity generated in PRP by exogenous arachidonate or by thrombin. Thrombin was used to liberate endogenous fatty acid from platelet phospholipids. PGH_3 (0.1–2 μM), PGI_2 (0.1–2 μM), PGH_1 (up to 5 μM), and dibutyryl cyclic AMP (up to 5 mM) did not alter the contractile activity formed by exogenous arachidonate (0.7 mM) in PRP (data not shown). However, very low levels of PGH_3 (0.3–0.5 μM) or PGI_2 (0.1 μM) inhibited arachidonate-induced PRP

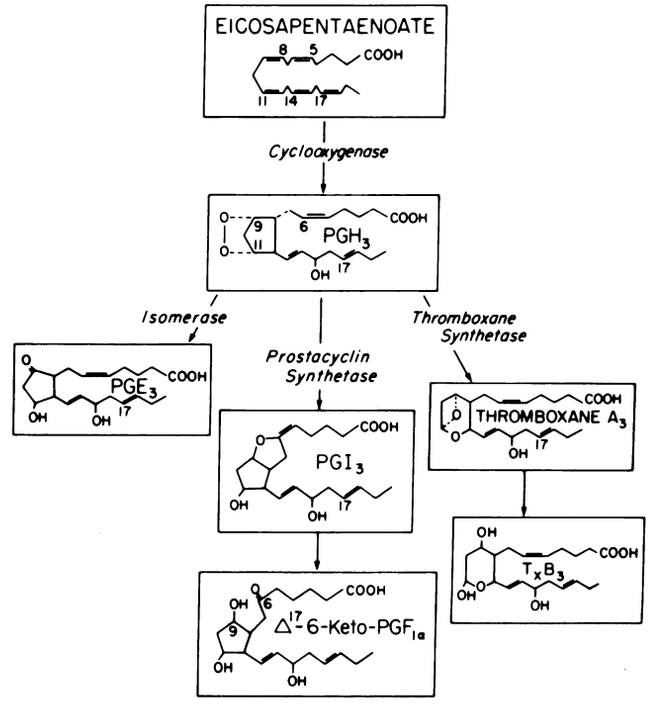

FIG. 6. Eicosapentaenoic acid metabolic pathway.

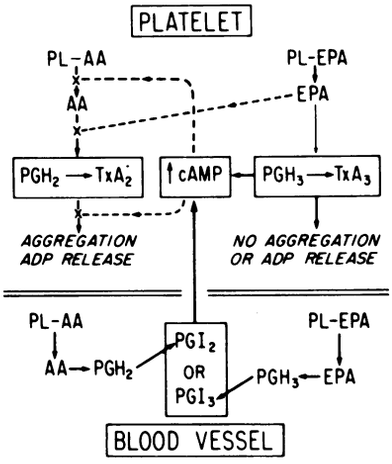

FIG. 7. PG metabolic pathways and sites of inhibition of the eicosapentaenoic acid (EPA) products on platelet aggregation. Tx, thromboxane; cAMP, cyclic AMP; AA, arachidonate; PL, phospholipid.

DISCUSSION

The synthetic pathway for the synthesis of the triene products is shown in Fig. 6. As a result of the additional Δ^{17}-unsaturation (the only structural difference between PGH_3 and PGH_2) present in PGH_3 or thromboxane A_3, platelet adenylate cyclase is stimulated and PRP aggregation and release are inhibited (Fig. 3). Conceivably, PGD_3 formation might also increase platelet cyclic AMP levels. On the other hand, PGI_2 and PGI_3 appear to exert similar effects both on vascular smooth muscle and on platelets.

PGH_3, PGH_1, and PGI_2, which increase platelet cyclic AMP, do not appear to alter platelet cyclooxygenase activity as evidenced by their inability to depress thromboxane A_2 formation from exogenous arachidonate. On the other hand, at concentrations that block PRP aggregation, PGH_3, PGH_1, and PGI_2 also interfere with the liberation (presumably by phospholipase activation) of fatty acids from platelet phospholipids as evidenced by their blockade of thrombin-induced fatty acid release and formation of rabbit aorta contractile activity in PRP (Fig. 4 *C* and *D*). The finding that compounds that increase cyclic AMP in PRP also inhibit lipase activity agrees with results in washed platelets (17, 24). Such agents thus would be anticipated to interfere with thrombin-induced platelet aggregation. This effect differs from that of aspirin on platelets because aspirin does not block thrombin-induced aggregation (25).

Platelet aggregation might be suppressed by vascular synthesis of PGI_3 (9). Dietary manipulations to increase the lipid content of eicosapentaenoic acid seems achievable because Greenland Eskimos have high levels of eicosapentaenoic acid and low levels of arachidonate in their serum lipids (9). However, the platelet lipids of the Eskimos have not been studied. We have in fact demonstrated that prostacyclin synthetase in isolated vascular segments will convert PGH_3 into PGI_3. It has been hypothesized that the balance between the formation of thromboxane A_2 by the platelets and prostacyclin (PGI_2) by the vascular walls regulates platelet aggregation and hemostatic plug formation *in vivo* (26). The present results indicate that vascular synthesis of PGI_3 is not obligatory to suppress platelet aggregation. Thus, if platelet phospholipids were rich in eicosapentaenoic acid instead of arachidonate, then agonists such as collagen, thrombin, or ADP which stimulate platelet phospholipase A_2 would be expected to release eicosapentaenoic acid and to initiate intrinsic PGH_3 and thromboxane A_3 synthesis. We have observed that the platelet cyclooxygenase is inefficient in converting eicosapentaenoic acid compared to arachidonate (Fig. 5*B*). However, the eicosapentaenoate effectively competes with arachidonate for platelet cyclooxygenase and lipoxygenase and thereby suppresses PGH_2 and thromboxane A_2 formation (Fig. 5*A*). In addition, any PGH_3 and thromboxane A_3 formed would increase platelet cyclic AMP concentration and thereby endogenously inhibit aggregation by pro-aggregatory molecules and concomitantly inhibit platelet phospholipase activity. A schematic illustration of the sites for triene inhibition of platelet arachidonate metabolism and aggregation is shown in Fig. 7. Such mechanisms, possibly in concert with vascular PGI_3 production, could be expected to decrease platelet aggregation. Dietary manipulation of tissue fatty acids is possible. The acylation process seems to be nonselective because numerous unsaturated fatty acids can readily be incorporated into the phospholipid pool available for PG biosynthesis (27). In light of the demonstration that eicosapentaenoate can be incorporated into platelet phospholipids, a partial substitution of eicosapentaenoate for arachidonate in platelets might be useful in decreasing thrombotic tendencies and myocardial infarction.

The authors are grateful for the excellent technical assistance provided by Angela Wyche and Sue Bronson. This work was supported by National Institutes of Health Grants HL-20787, SCOR-HL-17646, and AM20387 and Contract HV-72945 to H.S. and Grant NS-09667 to J.A.F.

1. Needleman, P., Minkes, M. & Raz, A. (1976) *Science* **193**, 163–165.
2. Raz, A., Minkes, M. S. & Needleman, P. (1977) *Biochim. Biophys. Acta* **488**, 305–311.
3. Needleman, P., Kulkarni, P. S. & Raz, A. (1977) *Science* **195**, 409–412.
4. Isakson, P. C., Raz, A., Denny, S. E., Pure, E. & Needleman, P. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 101–105.
5. Raz, A., Isakson, P. C., Minkes, M. S. & Needleman, P. (1977) *J. Biol. Chem.* **252**, 1123–1126.
6. Dusting, G. J., Moncada, S. & Vane, J. R. (1977) *Prostaglandins* **13**, 3–16.
7. Johnson, R. A., Morton, D. R., Kinner, J. H., Gorman, R. R., McGuire, J. C., Sun, F. F., Whittaker, N., Bunting, S., Salmon, J., Moncada, S. & Vane, J. R. (1976) *Prostaglandins* **12**, 915–928.
8. Needleman, P., Bronson, S. D., Wyche, A., Sivakoff, M. & Nicolaou, K. C. (1978) *J. Clin. Invest.* **61**, 839–849.
9. Dyerberg, J., Bang, H. O., Stoffersen, E., Moncada, S. & Vane, J. R. (1978) *Lancet* **i**, 117–119.
10. Sprecher, H. (1971) *Lipids* **6**, 889–894.
11. Gorman, R. R., Sun, F. F., Miller, O. V. & Johnson, R. A. (1977) *Prostaglandins* **13**, 11043–1056.
12. Moncada, S., Gryglewski, R. J., Bunting, S. & Vane, J. R. (1976) *Prostaglandin* **12**, 715–738.
13. Funk, M. O., Isaac, R. & Porter, N. A. (1976) *Lipids* **11**, 113–117.
14. Ferreria, S. H. & Vane, J. R. (1967) *Nature (London)* **216**, 868–873.
15. Vane, J. R. (1971) *Nature (London) New Biol.* **231**, 232–236.
16. Kulkarni, P. S., Roberts, R. & Needleman, P. (1976) *Prostaglandins* **12**, 337–353.
17. Minkes, M., Stanford, N., Chi, M., Roth, G. J., Raz, A., Needleman, P. & Majerus, P. W. (1977) *J. Clin. Invest.* **59**, 449–454.
18. Needleman, P., Raz, A., Ferrendelli, J. A. & Minkes, M. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 1716–1719.
19. Steiner, A. L., Parker, C. W. & Kipnis, D. M. (1972) *J. Biol. Chem.* **247**, 1106–1113.
20. Vargaftig, B. B. & Chignard, M. (1975) *Agents Actions* **5**, 137–144.
21. Salzman, E. W. & Weisenberger, H. (1972) *Adv. Cyclic Nucleotide Res.* **1**, 231–241.
22. Haslam, R. J. (1973) *Ser. Haematol.* **6**, 333–350.
23. Miller, O. V. & Gorman, R. R. (1976) *J. Cyclic Nucleotide Res.* **2**, 79–87.
24. Lapetina, E. G., Schmitges, C. J., Chandrabose, K. & Cuatrecasas, P. (1977) *Biochem. Biophys. Res. Commun.* **76**, 828–835.
25. Zucker, M. B. & Peterson, J. (1970) *J. Lab. Clin. Med.* **76**, 66–75.
26. Moncada, S. & Vane, J. R. (1978) *Br. Med. Bull.* **34**, 129–138.
27. Hseuh, W., Isakson, P. C. & Needleman, P. (1977) *Prostaglandins* **13**, 1073–1091.