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Analogues of Arachidonic Acid Used To Evaluate Structural Determinants of Prostaglandin Receptor and Enzyme Specificities

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SUMMARY

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Using ^{14}C-labeled fatty acids (19:4 ω6, 19:4 ω5, 21:4 ω6, and 21:4 ω7), radiochemical evidence of the formation of α-nor-, ω-nor-, α-homo-, and ω-homoprostaglandin endoperoxides (PGH_2), prostacyclins (PGI_2), and thromboxanes (TA_2) was obtained. Investigation of the biological activities of these compounds indicates that, although 21:4 ω6 is a substrate for cyclooxygenase and its endoperoxide, α-homo-PGH_2, is a substrate for prostacyclin synthetase and thromboxane synthetase, α-homo-PGH_2, α-homo-PGI_2, and α-homo-TA_2 are inactive on all receptors studied. In contrast, 19:4 ω5, precursor to ω-nor-PGs, and 19:4 ω6, precursor to α-nor-PGs, form endoperoxides which are not only substrates of prostacyclin synthetase and thromboxane synthetase, but also aggregate platelets and contract rabbit aorta spiral strips. Surprisingly, both α-nor-TA_2 and ω-nor-TA_2 are partial agonists at vascular smooth muscle receptors but, unlike their respective endoperoxides, do not aggregate washed human platelets. In contrast, 21:4 ω7 is converted to ω-homo-PGH_2 and ω-homo-TA_2, which aggregate platelets and are full agonists of vascular smooth muscle receptors. In addition to the radiochemical studies, the thromboxanes are identified by their lability in aqueous solution and the inhibition of their formation by the thromboxane synthetase inhibitor imidazole. Bovine aorta microsomes synthesize ω-nor-PGI_2, which is a partial agonist, and ω-homo-PGI_2, which is a full agonist when evaluated for the ability to relax bovine coronary artery spirals. Although we found radiochemical evidence for the synthesis of α-nor-PGI_2 and α-homo-PGI_2, these compounds appear to be biologically inactive. The prostacyclin synthetase inhibitor 15-hydroperoxy-arachidonic acid blocks the formation of α-nor-PGI_2, ω-nor-PGI_2, α-homo-PGI_2, and ω-homo-PGI_2 as measured by either radiochemical or biological assay.

INTRODUCTION

The use of arachidonic acid analogues differing in chain length or the positions of the double bonds permits the systematic study of structure-activity relationships for both PG[2] synthetic enzymes and PG receptors. The substrate specificity of cyclooxygenase has been studied extensively by van Dorp *et al.* (1–4), who observed the formation of PGs from positional isomers (shifted double bonds) or substituted fatty acids of 19–22 carbon atoms in chain length. Investigation of the biological activity of the PGs formed generally demonstrated that the compounds were of low potency, perhaps indicating that intestinal smooth muscle receptors were more specific than cyclooxygenase (1). However, these studies preceded identification of PGI_2 and thromboxanes as metabolites of PG endoperoxides.

We have previously studied 20:3 ω6[3] (8,11,14-dihomo-γ-linoleic acid) and 20:5 ω3 (IPA) (5–8). The fatty acid 20:3 ω6 lacks the 5—6 double bond necessary for formation of prostacyclin or thromboxane, but PGH_1 is a substrate for thromboxane synthetase, which catalyzes its conversion to 12-hydroxyheptadecadienoic acid (8). Studies of IPA metabolism are complicated by the fact

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[2] The abbreviations used are: PG, prostaglandin; PGI_2, prostacyclin; PGH_2, prostaglandin endoperoxide; IPA, 5,8,11,14,17-icosapentanoic acid; TA_2, thromboxane A_2; TB_2, thromboxane B_2; DDA, 2′,5′-dideoxyadenosine; HHT, 12-L-hydroxyheptadeca-5,8,10-trienoic acid.

[3] The fatty acid nomenclature gives the carbon atom number followed by the number of double bonds. Thus ω6 indicates that the first double bond is at carbon atom 6, counted from the terminal methyl group of the fatty acid.

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that IPA is a poor substrate for cyclooxygenase. When this step is bypassed by addition of PGH_3, the efficient coupling between cyclooxygenase and thromboxane synthetase is lost, resulting in the increased nonenzymatic PGH_3 breakdown which has plagued previous studies (8). We have evaluated the platelet metabolism of 19:4 ω5, 19:4 ω6, 21:4 ω6, and 21:4 ω7, which are the precursors of ω-nor-, α-nor-, α-homo-, and ω-homo-PGs, respectively. Radiochemical evidence permitted characterization of substrate specificities; in addition, we have studied the activities of the endoperoxides, prostacyclins, and thromboxanes formed at a variety of receptor sites.

MATERIALS AND METHODS

Materials. The fatty acids 19:4 ω5, 19:4 ω6, 21:4 ω6, and 21:4 ω7 as well as [1-^{14}C]-19:4 ω5 (12.2 Ci/mole), [1-^{14}C]-19:4 ω6 (24.5 Ci/mole), [1-^{14}C]-21:4 ω6 (14.2 Ci/mole), and [1-^{14}C]-21:4 ω7 (22 Ci/mole) were prepared by total organic synthesis (9). Arachidonic acid, [1-^{14}C] 20:4 ω6 (55 mCi/mmole), was purchased from Amersham/Searle Corporation, Arlington Heights, Ill. ^{14}C-Labeled endoperoxides of 19:4 ω5, 19:4 ω6, 21:4 ω6, and 21:4 ω7 were enzymatically synthesized with acetone/pentane powder of sheep seminal vesicles and purified as described (10). PG standards, $PGF_{2\alpha}$, PGE_2, PGD_2, PGA_2, PGI_2, 6-keto-$PGF_{1\alpha}$, and TB_2 were kindly supplied by John Pike, The Upjohn Company, Kalamazoo, Mich. DDA was purchased from P-L Biochemicals, Milwaukee, Wisc.

Platelet studies. Washed human platelet suspensions were prepared as previously described (11). Washed platelets (0.3 ml) were incubated with ^{14}C-labeled fatty acid for 15 min at 37°, acidified to pH 3.5 with 2 N formic acid, and extracted twice with 2 volumes of ethyl acetate. The extract was dried, applied with unlabeled PG standards to silica gel plates, and chromatographed in System C: $CHCl_3$-MeOH-acetic acid-water [90:8:1:0.8 (v/v)]. The PG standards were visualized by iodine staining. The radioactive peaks were detected on a Vangard model 940 scanner (Packard Instrument Company, Downers Grove, Ill.). The radioactive products were quantitated by scraping each zone and liquid scintillation counting.

The fatty acids and endoperoxides were also bioassayed for both the ability to aggregate washed human platelets and to contract vascular smooth muscle. The threshold doses of agonists which caused maximal aggregation were determined by direct addition of the fatty acids or purified endoperoxides to washed platelets pretreated with the adenylate cyclase inhibitor, DDA (12), and the thromboxane synthetase inhibitor, imidazole. Figure 1 illustrates the rationale behind this protocol. We studied the rate of the aqueous breakdown of the endoperoxides in imidazole-treated washed platelet suspensions at 37° to mimic the conditions of other experiments. Since the extent of platelet aggregation was dose-dependent, we assayed the endoperoxide remaining at any given time for its ability to aggregate DDA and imidazole-pretreated washed platelets. Since α-homo-PGH_2 has no measurable bioactivity, we were unable to determine its half-life.

Washed platelets and fatty acid were incubated at 37°

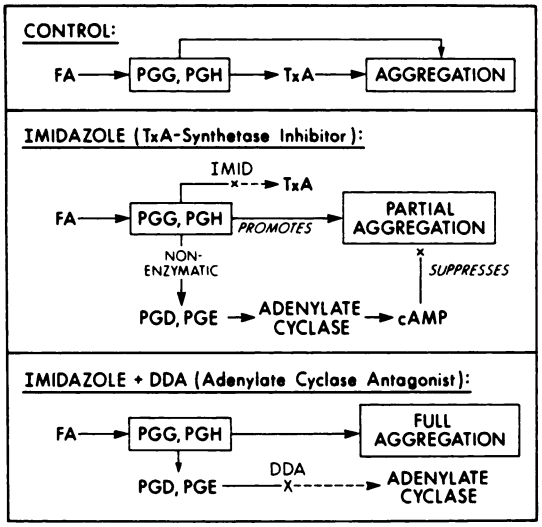

FIG. 1. *Schematic representation of the platelet metabolism of fatty acids and its pharmacological manipulation*

The *x* denotes the site of inhibition. *FA*, fatty acid; *TxA*, thromboxane A; *IMID*, imidazole.

for 30 sec, unless otherwise stated, and the suspension was injected over a superfusion cascade of rabbit aorta spirals which were simultaneously treated with indomethacin and a mixture of antagonists of epinephrine, serotonin, histamine, and acetylcholine (13).

Blood vessel studies. Bovine aorta microsomes were incubated with ^{14}C-labeled endoperoxides for 10 min at 37°, acidified to pH 3.5 with 2 N formic acid, and extracted twice with 2 volumes of ethyl acetate. The extract was chromatographed in the organic phase of ethyl acetate-trimethylpentane-acetic acid-water, 110:50:20:100 (v/v). PG standards co-chromatographed with the samples were visualized with iodine vapor. The position of radioactive material on the thin-layer chromatogram was determined by radioscanning and quantitated by scraping the zones and counting by liquid scintillation spectrophotometry.

Imidazole-treated washed human platelets and the fatty acids were used as an endoperoxide-generating system; bovine aorta microsomes, prepared as previously described (14), were included to convert the newly made endoperoxides to prostacyclins. The activities of the prostacyclins so formed were examined by bioassay on bovine coronary artery spirals in a superfusion cascade.

RESULTS

Metabolism of 19:4 ω5, 19:4 ω6, 20:4 ω6, 21:4 ω6, and 21:4 ω7 fatty acids. When ^{14}C-labeled fatty acids were incubated with human washed platelets at 37° for 15 min, labeled products were formed which co-chromatographed with authentic TB_2 standard. Additional products migrated with R_F values corresponding to those previously reported in the literature for HHT and for 12-hydroxyicosatetraenoic acid (for which no standards were available). Synthesis of TB_2 and HHT was blocked if the incubations were performed in the presence of 5 mM imidazole, an inhibitor of thromboxane synthetase. Counts in the TB_2 and HHT peaks accounted for 62% of

TABLE 1

^{14}C-Labeled fatty acid metabolism by washed platelets

Values are means ± standard error from three experiments determined as described under Materials and Methods.

Fatty acid	% Total counts on the plate[a]				
	T_xB_2	HHT	PG F,E,D	HETE	FA
20:4 ω6	30 ± 6	32 ± 4	9 ± 3	15 ± 3	3 ± 1
19:4 ω5	9 ± 2	7 ± 2	2 ± 0	30 ± 7	44 ± 10
19:4 ω6	14 ± 2	24 ± 3	4 ± 1	24 ± 2	28 ± 6
21:4 ω6	7 ± 2	11 ± 1	3 ± 0	69 ± 1	6 ± 3
21:4 ω7	28 ± 2	33 ± 7	2 ± 0	30 ± 6	8 ± 4
20:3 ω6	4 ± 1	78 ± 3	6 ± 1	10 ± 3	2 ± 0

[a] TxB_2, Thromboxane B_2; HETE, 12-hydroxyicosatetraenoic acid; FA, fatty acid.

the total counts on the plate for 20:4 ω6, 16% for 19:4 ω5, 38% for 19:4 ω6, 18% for 21:4 ω6, and 61% for 21:4 ω7, respectively (Table 1).

Since bovine aorta microsomes do not readily metabolize fatty acids, the fatty acids were converted to their respective endoperoxides and these were then incubated with bovine aorta microsomes at 37° for 10 min. Chromatography of the acid-lipid extract revealed labeled products from all four endoperoxides which co-migrated with authentic 6-keto-$PGF_{1\alpha}$ standard and whose synthesis was inhibited if 15-hydroperoxy-arachidonic acid (data not shown). We calculated that 43 ± 8% of the added PGH_2 was metabolized by prostacyclin synthetase as measured by 6-keto-$PGF_{1\alpha}$ formation, whereas 33 ± 7% of the ω-nor-PGH_2, 16 ± 2% of the α-nor-PGH_2, and 20 ± 4% of the α-homo PGH_2 were metabolized by prostacyclin synthetase ($n = 3$).

Effects of thromboxanes and endoperoxides upon platelet aggregation. We studied platelet aggregation to assess the bioactivity of the ω-nor-, α-nor-, α-homo-, and ω-homo-endoperoxides and thromboxanes. These compounds were compared with PGH_1 and PGH_3, as well as PGH_2 and TA_2 under the same conditions. Treatment of the platelets with imidazole, the thromboxane synthetase inhibitor which blocks the enzymatic conversion of endoperoxides to the C-17 hydroxy fatty acids and thromboxanes, allowed us to distinguish between the effects of the endoperoxides themselves, seen in imidazole-treated washed platelets, and the effects of their respective thromboxanes which were seen when thromboxane synthesis by the platelets was not inhibited (protocol shown in Fig. 1). We found that 19:4 ω5, 19:4 ω6, and 21:4 ω6 at doses up to 1 μg/0.3 ml failed to aggregate untreated washed platelets (Fig. 2). After pretreatment of the washed platelets with 100 mM DDA, an inhibitor of platelet adenylate cyclase (12), both 19:4 ω5 and 19:4 ω6 caused slight aggregation (Fig. 2). Surprisingly, when washed platelets were pretreated with 5 mM imidazole, thus increasing the endoperoxide concentration by inhibiting its rapid metabolism by thromboxane synthetase, 19:4 ω5 and 19:4 ω6 caused partial aggregation. The combination of DDA and imidazole pretreatment of washed platelets resulted in full aggregation by 19:4 ω6 and variable aggregation (50–100%) by 19:4 ω5 (Fig. 2). When it was added to untreated human washed platelets, 21:4 ω7 induced full aggregation. Neither DDA nor imidazole, in combination or alone, unmasked washed platelet aggregation by 21:4 ω6. Two lines of evidence indicated that the failure to aggregate with this latter fatty acid did not result from a failure to be metabolized by the platelet cyclooxygenase: (a) radiochemical studies demonstrated the formation of α-homo-TA_2, and (b) α-homo-PGH_2 also failed to aggregate platelets. This is in contrast to 20:5 ω3 (IPA), which is a poor substrate for platelet cyclooxygenase and so does not cause aggregation (8) whereas its endoperoxide (PGH_3) readily aggregates DDA-treated washed platelets (Table 2, ref. 7).

The threshold dose at which full (or maximal) aggregation occurred in DDA- and imidazole-pretreated washed platelets was determined for several fatty acids and their respective endoperoxides (Table 2). On the basis of these measurements, we assigned a rank order of potency for the fatty acids: arachidonic acid, 100%; 21:4 ω7, 20%; 19:4 ω6 and 20:3 ω6 approximately equipotent with 14% of the activity of arachidonic acid; and 19:4 ω5 as a partial agonist with 7% of the activity of arachidonic acid. The endoperoxides measured directly removed the effect of the differing affinities of the fatty acids for platelet cyclooxygenase and lipoxygenase. We assigned a

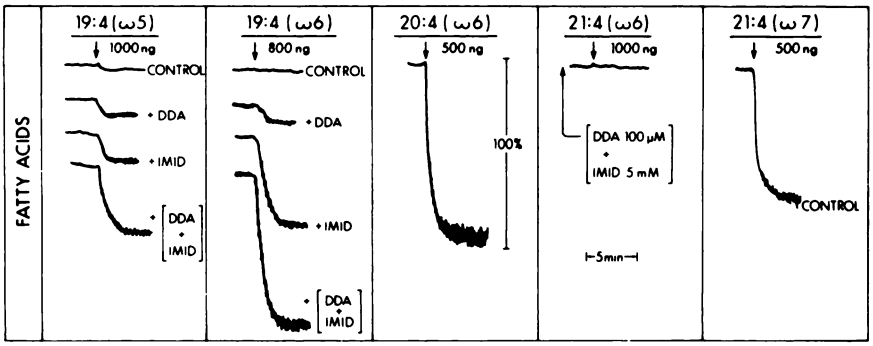

FIG. 2. *Effects of DDA and imidazole (IMID), in combination and alone, on aggregation of washed human platelets by fatty acids*

Aggregation was measured turbidometrically. DDA (100 μM) and imidazole (5 mM) were incubated with the washed platelets for 1 min prior to addition of agonists (*arrows*). Comparable results were obtained in two or three experiments.

TABLE 2

Fatty acid- and endoperoxide-induced platelet aggregation

Aggregation was studied in washed human platelets pretreated with 100 μM DDA and 5 mM imidazole. Either the fatty acid or the previously prepared and purified endoperoxide was added directly to the aggregation cuvette.

Fatty acid precursor	Threshold dose producing maximal aggregation	
	Fatty acid	Endoperoxide
	ng	*ng*
20:4 ω6	38 ± 8 (3)	35 ± 13 (5)
19:4 ω5	550 ± 50 (4)[a]	130 ± 30 (5)[b]
19:4 ω6	280 ± 80 (5)	35 ± 12 (5)
21:4 ω7	200 ± 0 (3)	25 (2)
21:4 ω6	NA (4)[c]	NA (3)[c]
20:3 ω6	270 ± 30 (3)	225 ± 25 (4)
20:5 ω6	NA (3)[c]	470 ± 80 (6)

[a] In two of four experiments, maximal aggregation was 50% of full aggregation.

[b] In all experiments, maximal aggregation achieved with this endoperoxide was 50% of full aggregation.

[c] No aggregation was seen under any conditions at any dose.

rank order of potency as aggregators of washed platelets for the endoperoxides as follows: PGH_2, 100%; α-nor-PGH_2 100%; ω-nor-PGH_2, 27%; PGH_1, 16%; PGH_3, 7%, and α-homo-PGH_2, inactive.

The requirement for DDA for full aggregation by α-nor- and ω-nor-PGH_2 suggested that platelet adenylate cyclase may be stimulated by nonenzymatic breakdown products of the endoperoxides. We investigated the rate and products formed by the non-enzymatic breakdown of endoperoxides. The half-lives of the endoperoxides in imidazole-treated washed platelets at 37° were similar: $t_{1/2}$ PGH_2, 2.7 min; $t_{1/2}$ ω-nor-PGH_2, 3.0 min; $t_{1/2}$ α-nor-PGH_2, 2.2 min; and $t_{1/2}$ ω-homo-PGH_2, 1.5 min. Complete degradation of the endoperoxides under these conditions produces comparable amounts of their respective E and D prostaglandins. Experiments are in progress to identify the inhibitory species and characterize their mode(s) of action.

Effects of thromboxanes and endoperoxides on vascular smooth muscle. In view of the observation that the 19:4 ω5- and 19:4 ω6-endoperoxides were more potent than their respective thromboxanes in inducing platelet aggregation, we compared these four compounds with regard to their ability to contract rabbit aorta spiral strips. In contrast to the platelet receptors, the receptors on vascular smooth muscle were more responsive to the α-nor- and ω-nor-thromboxanes than to their respective endoperoxides (Figs. 3 and 4). These thromboxanes (generated by incubation of washed platelets and fatty acid) shared with TA_2 the property of an extremely short half-life, whereas the endoperoxides, generated as in previous experiments by incubation of imidazole-treated washed platelets with fatty acids, were more stable (Fig. 3). We compared the relative potencies of endoperoxides (Fig. 4a) and thromboxanes (Fig. 4b) generated from arachidonic acid, 19:4 ω5, 19:4 ω6, 21:4 ω6, and 21:4 ω7 on rabbit aorta spirals. We used the stable analogue, cyclic ether endoperoxide (9,11-methanoepoxyendoperoxide) as a standard. Both α-nor- and ω-nor-thromboxane were partial agonists; their thresholds for rabbit aorta contraction were displaced to the right roughly one order of magnitude, while the maximal responses were less than 40% of that seen with TA_2. The ω-homo-TA_2 was a full agonist, with 13–28% the activity of TA_2. The α-homo-TA_2 gen-

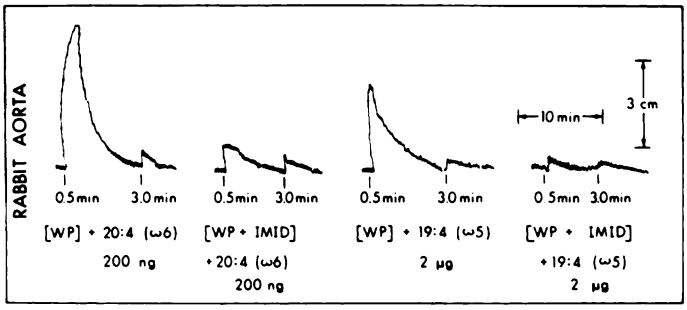

FIG. 3. *Bioassay of thromboxanes and endoperoxides formed by incubation of 20:4 ω6 (arachidonic acid) and 19:4 ω5 with 0.3 ml of washed human platelets*

Incubations of 0.5- and 3.0-min duration at 37° were tested on rabbit aorta spiral strips. Imidazole (*IMID*) (5 mM) was added to washed platelets (*WP*) just before addition of fatty acid.

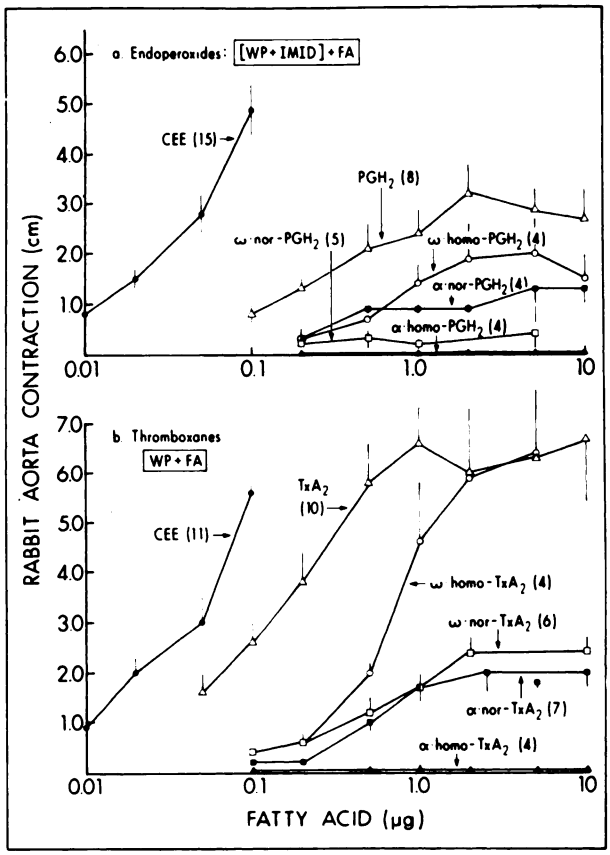

FIG. 4. *Dose-response curves for contraction of rabbit aorta spiral strips by cyclic ether endoperoxide [9,11-methanoepoxyendoperoxide (CEE)] or by fatty acids (FA) incubated with 0.3 ml of untreated washed human platelets (WP) (b) or imidazole-treated washed platelets (a) at 37° for 30 sec*

Values shown are means ± standard error; numbers in parentheses are numbers of aortas. *TxA*, Thromboxane A. CEE, ●; 20:4 ω6, △; 19:4 ω5, □; 19:4 ω6, ■; 21:4 ω6, ▲; and 21:4 ω7, ○.

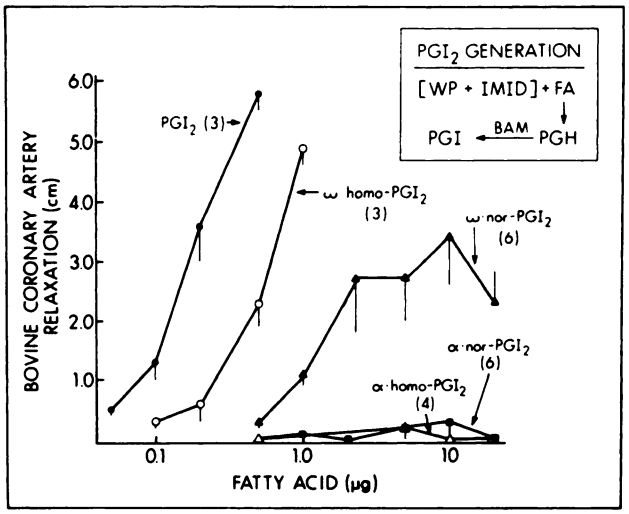

FIG. 5. *Dose-response curves for relaxation of bovine coronary artery strips by prostacyclins*

Prostacyclins were generated by incubation of 20:4 ω6, ●; 19:4 ω5, ▲; 19:4 ω6, ○; 21:4 ω6, ■; and 21:4 ω7, ○, with 0.3 ml of imidazole (*IMID*) (5 mM)-treated washed human platelets (*WP*) (endoperoxide-generating system) and bovine aorta microsomes (*BAM*) (PGI_2-generating system) for 30 sec at 37°. The dose-response curve of PGI_2 thus generated parallels that of PGI_2 standard. Values shown are means ± standard error; numbers in parentheses are numbers of coronary artery strips. *FA*, Fatty acid.

erated from 21:4 ω6 appeared to have no agonist activity. The α-nor- and ω-nor-endoperoxides also appeared to be partial agonists; the maximal response was 13–33% that of PGH_2. The endoperoxide ω-homo-PGH_2 was more active than the nor-endoperoxides, but whether it was a full or partial agonist was ambiguous. The endoperoxide α-homo-PGH_2 had no effect on the rabbit aorta.

Effects of prostacyclins on vascular smooth muscle. Radiochemical evidence suggested that prostacyclins were synthesized by bovine aorta microsomes from the endoperoxides prepared from each of the fatty acids under investigation (19:4 ω5, 19:4 ω6, 21:4 ω6, 21:4 ω7). We investigated the bioactivity of these compounds on bovine coronary artery spirals in a superfusion cascade. Imidazole-treated washed platelets and the fatty acids were used to generate the endoperoxides, which were converted to prostacyclins by added bovine aorta microsomes. Potent coronary relaxing substances were formed from 20:4 ω6, (PGI_2), 19:4 ω5 (ω-nor-PGI_2), and 21:4 ω7 (ω-homo-PGI_2), whereas α-nor- and α-homo-PGI_2 were inactive. Pretreatment of bovine aorta microsomes with 15-hydroperoxyarachidonic acid, a prostacyclin synthetase inhibitor, blocked formation of the coronary-relaxing substances. The dose-response relationships for the four prostacyclins were determined and compared with that for PGI_2 generated from arachidonic acid by the same procedure (Fig. 5). The dose-response curve for generated PGI_2 was parallel to that for authentic PGI_2 standard through the range of concentrations tested.

DISCUSSION

Enzyme and receptor specificities. In this report we have evaluated four fatty acids closely related to arachidonic acid for their abilities to form nor- and homo-prostaglandins and related compounds. The biological activities of these products at platelet and vascular receptors have been measured. One of the most striking observations is that the enzymes studied, cyclooxygenase, prostacyclin synthetase, and thromboxane synthetase, seem to have much broader specificities than do related receptors. Thus, the enzymes form a number of products with little or no biological activity.

Thromboxane receptors. This is the first report of discrimination between vascular and platelet thromboxane receptors by thromboxanes which are active at one site and not at the other. The thromboxanes α-nor-TA_2 and ω-nor-TA_2 have no measurable effect upon platelet aggregation; however, both are active as contractors of rabbit aorta. They appear to be partial agonists and produce maximal contractions which are only 40% of the maximal contraction attained by TA_2. The relative potency of these compounds was calculated by comparing the doses required to produce a fixed contractile response (1 or 2 cm); ω-nor-TA_2 is calculated to be 5–10% as potent as TA_2, and α-nor-TA_2 is 3–7%. Since there is less conversion of the two 19:4 fatty acids to nor-thromboxanes than of the arachidonic acid to TA_2, the relative potency might be 1.5 to 3 times greater. As partial agonists, the nor-thromboxanes have a potential as receptor antagonists; studies are currently in progress to explore this possibility.

The vascular thromboxane receptors also appear to have high specificity: although the α-nor- and ω-nor-thromboxanes retained some bioactivity, they were only partial agonists. However, ω-homo-TA_2 appears to be a full agonist, with 13–28% of the activity of TA_2; TA_3 is also a full agonist with 10% of the activity of TA_2 (5). The binding site of the receptor may be quite small, since addition of even 1 carbon atom on the α-chain causes complete loss of activity. The platelet thromboxane receptor appears to be even more constrained, since only TA_2, TA_3, ω-homo-TA_2, and the cyclic ether endoperoxides (9,11- and 11,9-methanoepoxyendoperoxide) induce platelet aggregation.

Endoperoxide receptors. Endoperoxides, on the other hand, appear to exhibit similar activity in binding to and eliciting a response on both vascular and platelet receptors. Thus ω-nor-PGH_2 has only modest activity on either blood vessels or platelets and furthermore shows the characteristics of a partial agonist at both receptor sites. α-nor-PGH_2 is more potent than ω-nor-PGH_2 on the platelet receptor and is apparently a full agonist. The dose-response curves of the nor-endoperoxides in contracting rabbit aorta are too flat to allow meaningful determination of relative potencies. As an expression of activity we compared the maximal contractions produced by α-nor-PGH_2 (33%) and ω-nor-PGH_2 (13%) with that produced by PGH_2 (100%). As on the platelet receptor, α-nor-PGH_2 appears to be more active than ω-nor-PGH_2 on the vascular smooth muscle receptor. Our studies here and previously indicate a rank order of potency for endoperoxides' activities on vascular receptors: PGH_2 > PGH_1 > PGH_3 > ω-homo-PGH_2 > α-nor-PGH_2 > ω-nor-PGH_2; α-homo-PGH_2 is inactive. Agonist potencies were calculated from the threshold doses producing maximal aggregation. The nor-endoperoxides have 35% (α-nor-

PGH_2) and 15% (ω-nor-PGH_2) of the potency of PGH_2, the same activities calculated on rabbit aorta. Although they are less active than PGH_1 and PGH_3 on the vascular receptor, the nor-endoperoxides are more potent than PGH_1 and PGH_3 on platelets.

These studies and our earlier work on PGH_3 and TA_3 (5) clearly indicate that the thromboxane and endoperoxide receptors are different. The dose-response curves on rabbit aorta for TA_2 and TA_3 are parallel to and distinct from those for PGH_1, PGH_2, and PGH_3 (5). Furthermore, our results show that the endoperoxides of a series may be active on the endoperoxide receptor whereas the corresponding thromboxanes are inactive on the thromboxane receptor.

Prostacyclin receptor. The vascular prostacyclin receptor appears to tolerate no alterations in the α-chain of the ligand. Of the six prostacyclins studied by this laboratory (ref. 6 and this paper) or others (15), the four which had biological activity had identical α-chains; addition or subtraction of a single carbon atom caused complete loss of activity at the vascular smooth muscle receptor. In contrast, prostacyclins altered by the addition or subtraction of a carbon atom or the addition of a double bond on the ω-chain retained at least partial biological activity. In fact, ω-homo-PGI_2 is reported to have 2–3 times the activity of PGI_2 at both vascular and platelet receptors (15); we observed 21% activity, perhaps due to differing quantities biosynthesized. Of course, these differences in potency may reflect differences in stability or distribution.

We attempted to evaluate the effects of ω-nor-, α-nor-, α-homo-, and ω-homo-prostacyclin at the platelet prostacyclin receptor. However, the bovine aorta microsomes used as a source of prostacyclin synthetase had intrinsic inhibitory activity. In addition, the high doses of endoperoxide required were complicated by the activity of endoperoxide in aggregating platelets and its ready breakdown to stable products which also exert an inhibitory effect via adenylate cyclase stimulation. Thus, it appears that investigation of the properties of the nor- and homo- prostacyclins must await their chemical synthesis.

In summary, we have differentiated between vascular and platelet thromboxane receptors, describing two thromboxanes active at only one site. The identification of ω-nor-TA_2 and α-nor-TA_2 as partial agonists at the vascular thromboxane receptor represents a promising first step toward the design of thromboxane receptor antagonists.

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