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Triene prostaglandins: Prostaglandin D_3 and icosapentaenoic acid as potential antithrombotic substances

(prostaglandin H_3/platelets/2′,5′-dideoxyadenosine)

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ABSTRACT **Addition of the 3-series fatty acid precursor (icosapentaenoic acid, IPA), its endoperoxide [prostaglandin (PG) H_3], or thromboxane A_3 to human platelet-rich plasma (PRP) does not result in aggregation of the platelets. In fact, preincubation of human PRP with exogenous PGH_3 actually inhibited aggregation by increasing platelet cyclic AMP concentrations. PGH_3 undergoes rapid spontaneous degradation to PGD_3 in human PRP. The PGD_3 so formed is adequate to account for the increase of platelet cAMP and inhibition of aggregation. Furthermore, addition of PGD-specific antisera to human PRP blocked the platelet inhibitory activity of exogenous PGH_3. PGD_3 has considerable potential as a circulating antithrombotic agent. Pretreatment of human PRP with the adenylate cyclase inhibitor 2′,5′-dideoxyadenosine blocked the increase of platelet cyclic AMP and the inhibition of aggregation normally produced by PGI_2, PGE_1, PGD_2, PGH_3, and PGD_3. Furthermore, the dideoxyadenosine unmasked a direct but moderate reversible aggregatory effect in response to the subsequent addition of PGH_3. Similarly, the dideoxyadenosine markedly enhanced the aggregation produced by exogenous PGH_2. IPA is readily incorporated into tissue lipids but proved to be a poor substrate for kidney, blood vessel, or heart cyclooxygenase. IPA was previously shown to be a poor substrate for platelet cyclooxygenase. IPA is readily deacylated from the renal phospholipid pool in response to bradykinin, a substance that also stimulates the release of arachidonic acid. A diet that relies primarily on cold-water fish, as in the case of the Greenland Eskimos, lowers endogenous arachidonic acid and markedly increases the IPA content of tissue lipids. Thus, because IPA has the potential to act as an antagonist with arachidonic acid for platelet cyclooxygenase and lipoxygenase, the simultaneous release of IPA could suppress any residual arachidonic acid conversion to its aggregatory metabolites.**

The normal 2-series prostaglandin (PG) family is derived from arachidonic acid (AA; 5,8,11,14-icosatetraenoic acid). AA and its metabolites PGH_2 and thromboxane A_2 are potent stimulators of platelet aggregation. The fatty acid precursor of the 3-series PG family, 5,8,11,14,17-icosapentaenoic acid (IPA), can be enzymatically converted by sheep cyclooxygenase into the PG endoperoxide PGH_3 (1, 2). Purified PGH_3 in turn is converted by the appropriate enzyme source into thromboxane A_3 or Δ^{17}-prostacyclin (PGI_3) (2). The 3-series endoperoxide and thromboxane are less potent contractile agents on rabbit thoracic aorta strips than the corresponding 2-series compounds (i.e., PGH_2 and thromboxane A_2) (1), and similarly PGI_3 relaxes isolated coronary arterial strips but is less potent than PGI_2 (3). Surprisingly, the 3-series fatty acid (IPA), endoperoxide (PGH_3), and thromboxane (thromboxane A_3) do not induce aggregation in human platelet-rich-plasma (PRP) (1) and, furthermore, preincubation of human PRP with exogenous PGH_3 or thromboxane A_3 inhibits aggregation with conventional stimuli (e.g., PGH_2, AA, ADP, thrombin) by elevating platelet cyclic AMP levels (2). The possible utility of substituting IPA (or its precursor 9,12,15-linolenic acid) for AA (or its precursor 9,12-linoleic acid) to achieve an antithrombotic diet emerged from the observation of Dyerberg *et al.* (4) that Greenland Eskimos, whose diet consists primarily of cold-water fish (high IPA content), have a bleeding tendency and have increased IPA and depressed AA concentrations in their blood lipid fraction. These authors proposed that the bleeding disorder resulted from the vascular synthesis of PGI_3. In order to further test the hypothesis that platelet aggregation could be depressed by IPA and its metabolites, we planned to initiate feeding experiments substituting linolenic acid for linoleic acid in the diet of laboratory animals. During control experiments we discovered that exogenous PGH_3 did not inhibit aggregation in rat or rabbit PRP; in fact, the PGH_3 exhibited weak aggregatory activity. This observation was reminiscent of the finding that PGD_2 inhibits aggregation only in human platelets (5). We therefore tested the possibility that PGD_3 (formed by the nonezymatic degradation of PGH_3) was the mediator of the increase of cyclic AMP concentrations that caused the inhibition of aggregation in human PRP. In addition, we pursued investigations designed to develop an insight into the mechanism whereby IPA interferes with human platelet aggregation.

MATERIALS AND METHODS

Materials. [1-^{14}C]IPA, 7.2 Ci/mol (1 Ci = 3.7×10^{10} becquerels), was prepared by total organic synthesis (6). [^{14}C]PGH_3 and [^{14}C]PGH_2 were enzymatically synthesized and purified with acetone/pentane powder of sheep seminal vesicles as described (1, 7). PGD_3 was enzymatically synthesized from unlabeled IPA (Nu Chek Prep., Elysian, MN) containing [^{14}C]IPA marker by employing the sheep seminal vesicle microsomes in the presence of 1 mM reduced glutathione and 1.2 mM epinephrine. The PGD_3 was separated on a silicic acid (Unisil, Mallinckrodt) column by sequential elution with ethyl acetate/methanol 1:4 and 1:3 (vol/vol); the remaining zones were eluted with methanol. The methanol fraction was concentrated under reduced pressure and chromatographed on silica gel thin-layer chromatography preparative plates in the A9 solvent system (ethyl acetate/acetic acid/2,2,4-trimethylpentane/water, 110:20:50:100, vol/vol). The PGD_3 zone (PGD_3 comigrated with authentic PGD_2) was scraped and extracted with methanol. The purified PGD_3 exhibits the same chromatographic mobility (in multiple solvent systems) and chemical properties as authentic PGD_2.

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Abbreviations: PG, prostaglandin; IPA, 5,8,11,14,17-icosapentaenoic acid; AA, 5,8,11,14-icosatetraenoic acid (arachidonic acid); PRP, platelet-rich plasma; ddA, 2′,5′-dideoxyadenosine.

An antiserum that binds PGD_2 was prepared by previously described methods (8). The antiserum specificity was determined under standard radioimmunoassay conditions. The percentages of crossreaction with heterologous PGs were: PGE_2 (0.002%), $PGF_{2\alpha}$ (0.2%), 6-keto-$PGF_{1\alpha}$ (0.01%), thromboxane B_2 (3.0%), PGD_1 (6.0%), $PGF_{1\alpha}$ (0.1%), 13,14-dihydro-$PGF_{2\alpha}$ (0.1%), 13,14-dihydro-15-keto-PGE_2 (0.03%), 13,14-dihydro-15-ketothromboxane B_2 (0.7%).

PG standards, PGE_2, PGD_2, PGA_2, prostacyclin (PGI_2), 6-keto-$PGF_{1\alpha}$, PGE_3, and thromboxane B_2 were kindly supplied by John Pike from Upjohn. The 2',5'-dideoxyadenosine was purchased from P-L Biochemicals.

Platelet Experiments. Citrated human PRP or washed human platelet suspensions were prepared as described (9). Platelet cyclic AMP concentrations (at 60 sec after addition of the test compound in the PRP) were determined by radioimmunoassay as described (10). Inhibition of platelet aggregation was studied by incubating the various PGs in PRP for 1 min prior to the addition of AA.

The [^{14}C]AA (1 μg, 300,000 cpm) was incubated with washed platelets (0.4 ml) at 37°C for 15 min, acidified to pH 3.5 with 2 M formic acid, and extracted twice with 2 vol of ethyl acetate. The extract was dried and applied with unlabeled PG standards to silica gel plates. The solvent system was benzene/dioxane/acetic acid, 60:30:3 (vol/vol). The PG standards were visualized by iodine staining. The radioactive peaks were detected on a Vangard Scanner (Packard). In fatty acid mixing experiments, the unlabeled IPA was mixed with the [^{14}C]AA and the reaction was started by adding the platelets.

Blood Vessel–Fatty Acid Incubations. Rabbit thoracic aorta was cut into fine rings (total weight of 10–15 rings was 120 mg) and incubated with the ^{14}C-labeled fatty acid (AA or IPA) (10^6 cpm) in 10 ml of 100 mM potassium phosphate buffer, pH 7.8, at 37°C for 1 hr. The media were acidified, extracted, and chromatographed in the organic phase of the A9 solvent system. The lipid of the aorta rings was extracted with chloroform/methanol (2:1, vol/vol), concentrated, and applied with unlabeled standards to silica gel G plates. The solvent system employed was chloroform/methanol/ammonium hydroxide, 65:35:5 (vol/vol).

Perfused Kidney Experiments. The method of incorporating the radioactive AA or IPA into the renal lipids has been described in detail (11). Briefly, 5 μCi of ^{14}C-labeled fatty acid, prepared as the sodium salt in saline (pH 9), was infused through the renal vascular bed of the perfused kidney for 20 min. The ^{14}C-labeled fatty acid incorporated into the endogenous tissue lipids (primarily into phospholipids) can be released in the form of ^{14}C-labeled PGs (in the case of AA) by agents that stimulate PG biosynthesis (12). Fatty acid-free albumin was included in the perfusion media to trap the ^{14}C-labeled fatty acid released by agonist stimulation (13). The acidified organic extract of the renal venous effluent was analyzed on silica gel plates in the organic phase of the A9 solvent system.

Table 1. Comparison of the spontaneous degradation of the PG endoperoxides (PGH_2 and PGH_3) and their ability to inhibit human PRP aggregation

PG	$t_{1/2}$ of endoperoxide in PRP, sec	Total PGD formed from endoperoxide, %	IC_{50} for human PRP aggregation, ng/ml	
			PGH	PGD
2-Series	350 ± 35	24 ± 6	*	20 ± 3
3-Series	90 ± 8	20 ± 2	85 ± 20	7 ± 2

The degradation half time ($t_{1/2}$) at 37°C was determined by testing the potency of the endoperoxide to contract isolated rabbit thoracic aorta strips (superfused with Krebs–Henseleit medium). An acetone solution of the endoperoxide was dried in a stream of N_2, reconstituted in PRP prewarmed to 37°C, and after various incubation periods was tested by injection directly over the blood vessel strip. The percentage conversion to PGD at 100% breakdown was determined by incubating either [^{14}C]PGH_2 or [^{14}C]PGH_3 (30,000 cpm) in PRP for 30 min or 10 min, respectively, at 37°C, followed by acid lipid extraction and thin-layer chromatography. Inhibition of aggregation was determinated by preincubating the respective endoperoxide or PGD for 1 min with PRP prior to addition of AA (500 μg/ml). Figures are mean ± SEM.

* PGH_2 actually aggregates PRP.

RESULTS

Plasma degradation of PGH_3 to PGD_3 and its effect on aggregation

Preincubation of exogenous PGH_3 for 1 min with human PRP resulted in a concentration-dependent inhibition of aggregation by AA, with a dose causing 50% inhibition (IC_{50}) of 85 ng/ml (Table 1). There was a concomitant 40 ± 15% increase (mean ± SEM) in the platelet cyclic AMP concentration. At the IC_{100} (650 ng/ml) for PGH_3, the cyclic AMP concentration was 503 ± 108% of the unstimulated control values. PGD_3 was considerably more potent in inhibiting aggregation, with an IC_{50} of 7 ng/ml, which elevates platelet cyclic AMP concentrations 39 ± 17% ($n = 5$), whereas the IC_{100} (33 ng/ml) resulted in a 4-fold increase in cyclic nucleotide. PGH_2, of course, readily induced platelet aggregation, but PGD_2 inhibited aggregation, although the IC_{50} was larger (20 ng/ml) than for PGD_3. We determined the total PGD that might be formed from endoperoxide by incubating ^{14}C-labeled PGH_3 or PGH_2 with PRP. The rate of

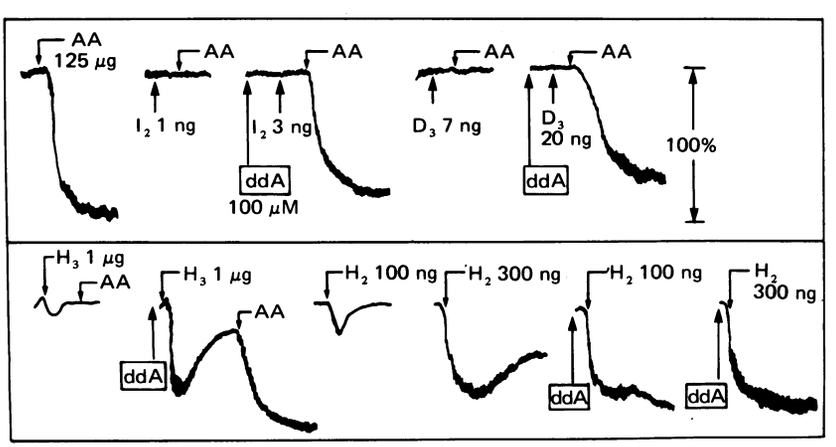

FIG. 1. Effect of ddA on aggregation in human PRP. Aggregation was measured turbidimetrically. I_2, PGI_2 (prostacyclin); D_3, PGD_3; H_3, PGH_3; and H_2, PGH_2. The ddA was preincubated with the PRP for 1 min prior to agonist addition. The PGI_3, PGD_3, or PGH_3 preincubation was for an additional 1 min. Comparable results were obtained in five experiments. The same amounts of AA and ddA were used in each case.

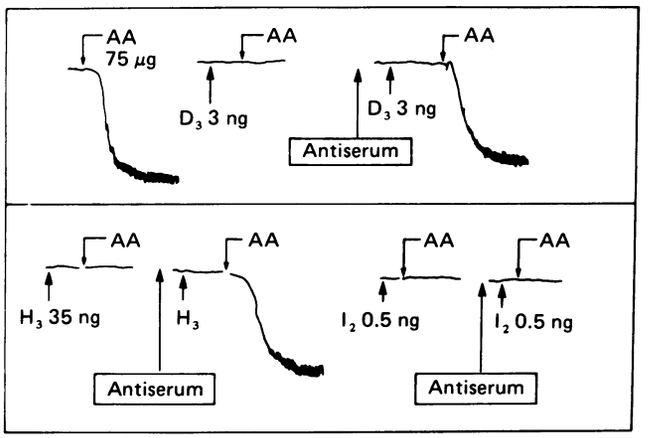

FIG. 2. Effect of PGD antiserum (20 μl, 2 min at 37°C) on the inhibition of human platelet aggregation by PGD_3 (D_3), PGH_3 (H_3), or PGI_2 (I_2).

endoperoxide degradation in plasma was determined by the loss of rabbit aorta contractile activity in PRP with time (Table 1). Although comparable amounts of PGD are generated from each endoperoxide, PGH_3 is much more rapidly (4-fold) degraded than PGH_2. On the basis of these data, a 1-min incubation of 85 ng/ml PGH_3 (IC_{50}) in PRP would result in the formation of PGD_3 at 7 ng/ml, which closely approximates the IC_{50} of authentic PGD_3. Although PGH_2 is aggregatory, presumably by direct action on the platelets, in 1 min, 100 ng of the endoperoxide would be converted to enough PGD_2 (8 ng) to achieve 50% inhibition of aggregation.

In another set of experiments, we varied the time of preincubation of either PGH_3 or PGD_3 with PRP prior to initiating aggregation with exogenous AA. Exogenous PGD_3 immediately inhibited aggregation and increased platelet cyclic AMP, whereas a precincubation time of 30 sec was required before PGH_3 suppressed aggregation.

Effect of endoperoxides after inhibition of platelet adenylate cyclase

Haslam *et al.* (14) have demonstrated that 2′,5′-dideoxyadenosine (ddA) inhibits the increase of platelet cyclic AMP by PGE_1 and that ddA does not alter AA-induced aggregation. Preincubation of human PRP with ddA blocked the increase of platelet cyclic AMP and the inhibition of aggregation produced by PGI_2 or PGD_3 (Fig. 1). ddA had no effect on basal (13.5 pmol/ml of PRP) cyclic AMP concentrations, but prevented the increase produced by prostacyclin at 10 ng/ml (89 pmol/ml of PRP) or PGD_3 at 60 ng/ml (81.6 pmol/ml of PRP). The prevention of the stimulation of platelet adenylate cyclase by ddA did in fact unmask a direct but moderate and reversible aggregatory effect of PGH_3 as well as block the ability of this endoperoxide to inhibit platelet aggregation (Fig. 1). However, even with ddA, higher doses of PGH_3 did not produce complete irreversible aggregation. The ddA pretreatment also demonstrated that although PGH_2 is an aggregatory stimulus its direct effect on platelets is compromised by an increase of platelet cyclic AMP, presumably induced by the spontaneously generated PGD_2. Thus, PGH_2 at 300 ng/ml was only a weak stimulator of aggregation (Fig. 1) under conditions that would have generated PGD_2 at 25 ng/ml (Table 1), and ddA pretreatment dramatically enhanced the aggregation induced by PGH_2. It is now clear that any previous experiment that utilized exogenous endoperoxide including PGH_2 (15, 16) was most likely compromised by the spontaneous generation of PGD_2 (Table 1, Fig 1). We also observed that the inhibition of platelet

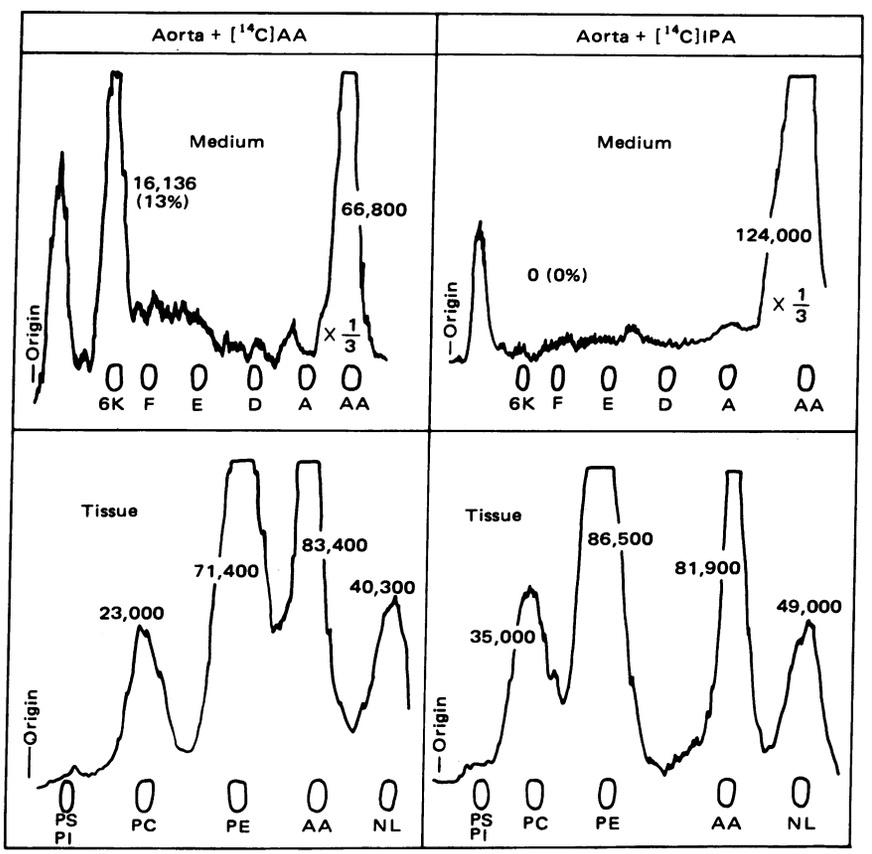

FIG. 3. Acylation and metabolism of [^{14}C]AA or [^{14}C]IPA by rabbit thoracic aorta rings. Blood vessel rings were incubated with ^{14}C-labeled fatty acid and the labeled PG metabolites were extracted from the medium and the labeled lipids from the tissues. Unlabeled standards were added to the concentrates and were applied to the plate. After development in the appropriate solvent system the standards were visualized with iodine vapor, and the radioactivity (vertical scale) was determined on the Vangard scanner. The peaks were scraped off and their measured radioactivities are indicated on the radioscans. 6K, 6-keto-$PGF_{1\alpha}$; F, $PGF_{2\alpha}$; E, PGE_2; D, PGD_2; A, PGA_1; PS, phosphatidylserine; PI, phosphatidylinositol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; and NL, neutral lipids. The conventional silicic acid thin-layer plates do not resolve compounds by their degree of unsaturation; i.e., they do not separate 2-series PGs from 3-series PGs.

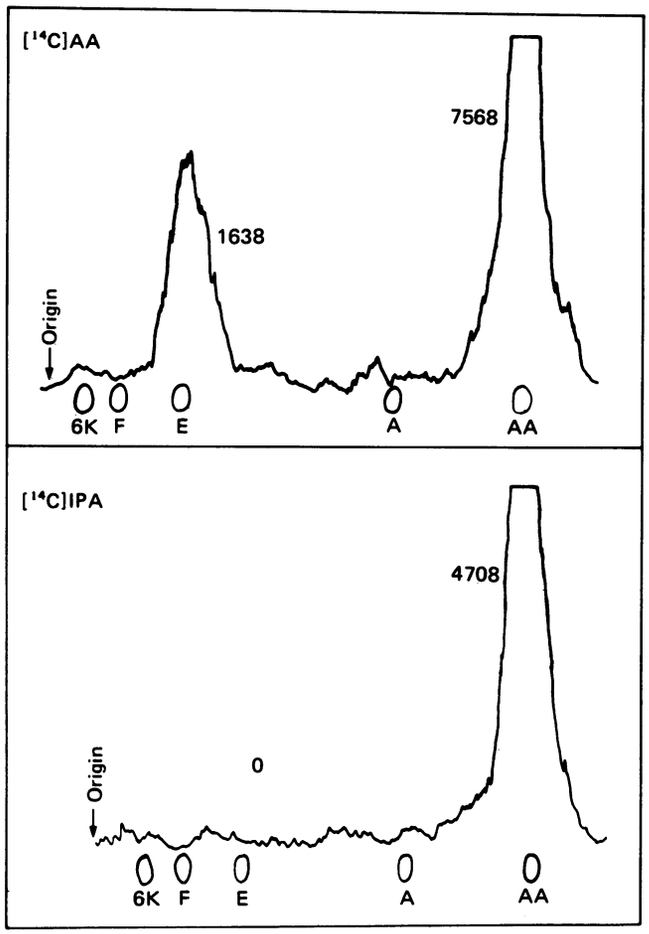

FIG. 4. Release into the media of ^{14}C-labeled products from bradykinin-stimulated hydronephrotic rabbit kidneys. The kidneys had their phospholipids labeled by slow infusion of [^{14}C]AA or [^{14}C]IPA. Six hours later 50 ml of renal venous effluent was collected for extraction and analysis of the basal sample. The kidneys were then stimulated by a bolus injection of bradykinin (3 μg) and 50 ml of the effluent was acidified, extracted, and chromatographed (benzene/dioxane/acetic acid, 60:30:3 vol/vol). Similar results were obtained in three repetitions of this experiment. The various zones of the chromatogram were scraped, eluted, and assayed for radioactivity, and the values are indicated on the radioscan. Abbreviations are as for Fig. 3.

aggregation by PGE_1 and PGD_2 was prevented by ddA (not shown). Furthermore, in rabbit PRP neither PGD_2 nor PGD_3 increased platelet cyclic AMP or inhibited aggregation, and addition of PGH_3 (12 μg/ml) caused a reversible aggregation not influenced by ddA.

PGD antisera

By utilizing antiserum specifically directed against PGD we were able to demonstrate that the platelet inhibitory activity of PGH_3 was due to the spontaneous degradation to PGD_3 (Fig. 2). The antiserum was preincubated with PRP and was able to block the effect of the threshold inhibitory doses of authentic PGD_3 as well as PGH_3. Furthermore, preincubation with antiserum blocked the effect of a threshold inhibitory dose of PGH_3, showing conclusively that the inhibition was due not to the PGH_3 itself but to PGD_3. The dose of PGH_3 used was far below the amount needed to cause aggregation (Fig. 1). The specificity of the antiserum is emphasized by its inability to interfere with the inhibition of platelet aggregation by PGI_2.

Tissue incorporation and metabolism of IPA

PGD formation by platelets would be expected to occur only upon the addition of an exogenous source of PG endoperoxide, because endogenously formed endoperoxide is almost exclusively converted to thromboxane before any significant breakdown to D and E can occur (17). Thus, evidence that high intrinsic IPA could interfere with platelet aggregation would either consist of the demonstration of adequate levels of PGI_3 production by the vasculature or documentation that IPA is readily incorporated and released from tissue lipids and that this fatty acid competes with AA. We can readily demonstrate that both [^{14}C]IPA and [^{14}C]AA are incorporated into the rabbit aorta phospholipid pool (Fig. 3). However, under incubation conditions that result in a 10–20% conversion of AA by blood vessel cyclooxygenase, there was no detectable conversion of [^{14}C]IPA to Δ^{17}-6-keto-[^{14}C]$PGF_{1\alpha}$ (Fig. 3). Similarily, exogenous [^{14}C]IPA proved to be a poor substrate for heart (not shown) and kidney (Fig. 4) cyclooxygenase, although it is readily incorporated into the tissue lipids.

On the other hand, we found that IPA is readily released from tissue lipids by the same conditions that activate the release of AA. We infused isolated perfused hydronephrotic rabbit kidneys with either [^{14}C]AA or [^{14}C]IPA in order to label the renal phospholipid pool. These prelabeled kidneys were stimulated by a bolus injection of bradykinin (3 μg), and the venous effluent was collected, extracted, and chromatographed (Fig. 4). The peptide stimulation resulted in the release of both ^{14}C-labeled fatty acids by phospholipase activation, but only the AA was converted to a PG metabolite (Fig. 4). Similarly, hydronephrotic rabbit kidneys that had their phospholipids prelabeled with both [^{14}C]IPA and [^{14}C]AA released both fatty acids simultaneously when stimulated with bradykinin (not shown). Whereas the enzymatic conversion of IPA could not be demonstrated in rabbit kidney homogenates, the IPA did inhibit the renal metabolism *in vitro* of [^{14}C]AA. We previously obtained (2) similar results—thrombin could readily release [^{14}C]IPA from prelabeled platelets—but this fatty acid was a poor substrate for platelet cyclooxygenase. The only tissue source that we have tested that readily converts IPA to PGs is ram seminal vesicle (data not shown).

DISCUSSION

We initially rejected the possibility that the inhibition of platelet aggregation by PGH_3 (or thromboxane A_3) could be mediated by PGD_3 because the 3-series endoperoxide, thromboxane, and prostacyclin were all less active on vascular smooth muscle than their 2-series counterparts (1–3). Furthermore, we also observed that PGE_3 was 1/10th–1/30th as active as PGE_2 as a contractile agent on nonvascular and vascular smooth muscle (data not shown). It is therefore quite surprising that PGD_3 is so active on human platelets and that the PGH_3 breakdown to PGD_3 is much more rapid than anticipated. As the above calculation shows, the PGD_3 spontaneously generated during the 1-min preincubation of PGH_3 with human PRP is adequate to quantitatively account for the increase of platelet cAMP and inhibition of aggregation by the endoperoxide. This observation was confirmed with the PGD antiserum, which prevented the PGD_3 and PGH_3 inhibition of aggregation.

The generation of the D prostaglandins described above reflects the nonenzymatic breakdown of exogenously added endoperoxide and does not normally occur when platelet PG synthesis is initiated with fatty acid, because intrinsically generated endoperoxide is so efficiently coupled to the thromboxane synthetase that there is little, if any, detectable PGD or PGE formation (17, 18).

These experiments seem to preclude the possible intrinsic platelet role of PGH_3, thromboxane A_3, or PGD_3 as an inhibitor of aggregation. It is apparent that exogenous PGD_3 has considerable potential as a therapeutic agent because it is a potent inhibitor of platelet aggregation, and this substance therefore warrants expanded investigation. PGD_2 proves to be a very poor substrate for pulmonary PG dehydrogenase, with a K_m that is 100 times higher than that for PGE_2 and a V_{max} that is very low (19). Exogenous PGD_3 or an appropriate analog should therefore be tested to determine if it is poorly degraded in transit across the lung, thereby providing a circulating antithrombotic agent. Furthermore, if the biological data obtained with the other 3-series products is a useful indication, then PGD_3 might be expected to have minimal effects on vascular and nonvascular smooth muscle at antithrombotic doses. Thus, PGD_3 could possibly avoid the profound vasodilator side effects of PGI_2 (20) as well as the diarrhea and gastrointestinal side effects induced by the conventional PGs (21).

Dyerberg *et al.* (4) suggested that the Eskimo bleeding disorder could be the result of the vascular synthesis of PGI_3. In their experiments, isolated blood vessels were repeatedly washed until the bathing medium no longer contained an inhibitor of platelet aggregation. The blood vessels were then powdered in liquid nitrogen and incubated with unlabeled IPA and found to again inhibit platelet aggregation. This latter effect was inhibited by pretreating the blood vessel with indomethacin. The problem with these experiments is that they lack any direct chemical proof of formation of PGI_3 and they do not preclude the possibility that the homogenization technique releases intrinsic AA for the vascular synthesis of PGI_2, which could then inhibit platelet aggregation. In fact, we find that incubation of [^{14}C]IPA with intact rabbit aorta rings does not lead to [^{14}C]PGI_3 production although the IPA is readily incorporated into the vascular lipid pool (Fig. 3). Under comparable incubation conditions up to 20% of the [^{14}C]AA is converted to [^{14}C]PGI_2. Similarly, we have been unable to demonstrate detectable conversion of IPA to PGI_3 by perfused hearts or kidneys, again suggesting that this fatty acid is a poor substrate for oxygenation by the cyclooxygenase.

We can demonstrate efficient incorporation of IPA into tissue lipids. We have also shown in platelets (2) and intact perfused kidneys (Fig. 4) that selective stimulation (thrombin and bradykinin, respectively) will readily lead to deacylation of the prelabeled lipid and release IPA. Previous experiments in platelets (22) and kidneys (12) have demonstrated that agonist stimulation is selective in its fatty acid release, releasing AA and IPA but no other fatty acids (including oleate, linoleate, and palmitate). No apparent distinction between IPA and AA release from tissue lipids exists. Because IPA acts as a competitive antagonist for AA (2), the simultaneous release of these fatty acids in platelets should effectively suppress AA conversion to its aggregatory metabolites. The simultaneous decrease in endogenous AA and increase in IPA that must result from a diet of the type that the Greenland Eskimos ingest would be anticipated to markedly reduce the generation of PGH_2 and thromboxane A_2. If these suggested mechanisms in fact explain the Eskimo bleeding tendency, then it should also be expected that there is little if any vascular PGI_2 or any other PG production (i.e., there is a PG deficiency). Obviously, it would be valuable to ascertain the total urinary PG production in this population. Indeed, these subjects would be useful to study in those conditions believed to be mediated by intrinsic PG synthesis (e.g., inflammation, ulcers, renal disease, etc.). The more likely expression of a major deficiency of intrinsic PG would be anticipated in pathological rather than physiological situations.

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