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Arachidonate Metabolism in Vascular Disorders

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Introduction

Many reactions including thrombosis, inflammation, wound healing, and allergy are modulated by oxygenated metabolites of arachidonate and related polyunsaturated fatty acids that are collectively termed eicosanoids. These mediators include prostaglandins, thromboxanes, leukotrienes, hydroxylated derivatives of arachidonate, and probably other oxygenated metabolites not yet discovered. In most instances, these mediators are not required to produce a response but rather modulate its intensity (either stimulate or inhibit). Thus, platelets will aggregate and form a hemostatic plug in response to thrombin, even when thromboxane A_2 production is inhibited; however, thromboxane A_2 will stimulate platelet plug formation. When thromboxane A_2 production is blocked, a mild hemostatic defect is produced as evidenced by prolongation of the skin bleeding time. Many eicosanoids are labile, producing their effect for only seconds to minutes. Interference with eicosanoid production is the basis for many therapeutic agents, including a number of analgesics, antiinflammatory drugs, and antithrombotic agents. Production of eicosanoid mediators is controlled by cellular mechanisms for the uptake and release of arachidonate from cellular phospholipids. When stimulated by a hormone, coagulation factor, or other agonist for which a particular cell has receptors, hydrolases release arachidonate from complex lipids. Arachidonate is then converted to an eicosanoid that is characteristic for the particular cell type.

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Role of eicosanoids in platelet function

The major products of arachidonate metabolism in platelets are a 12-hydroxy metabolite of arachidonate, which is a molecule of unknown function, and thromboxane A_2 (1), which is a labile inducer of platelet aggregation and a potent vasoconstrictor. Aspirin inhibits thromboxane production by covalent acetylation of the active site of cyclooxygenase (2), the enzyme that produces the cyclic endoperoxides, prostaglandins G_2 and H_2 (PGG_2 and PGH_2),[1] as shown in Fig. 1. Acetylation permanently inactivates the enzyme, and since platelets are incapable of protein synthesis, they cannot produce new, active enzyme. The aspirin effect lasts for the life of the platelet (7–10 d) so that repeated dosage leads to cumulative inhibition of cyclooxygenase. Daily administration of very small amounts of aspirin, much less than those required for other therapeutic actions of the drug, completely inactivate platelet cyclooxygenase (3).

Arachidonate metabolism by endothelium

Kulkarni et al. (4) demonstrated that blood vessels convert arachidonate to a potent vasodilator, the production of which is inhibited by aspirin. The substance was identified by Johnson et al. (5) as prostaglandin I_2 (PGI_2), a previously discovered arachidonate metabolite of unknown function (6). PGI_2, or prostacyclin, is a labile, potent inhibitor of platelet aggregation (7), which

[1] Abbreviations used in this paper: PGD_2, PGH_2, PGI_2, prostaglandins D_2, H_2, and I_2, respectively.

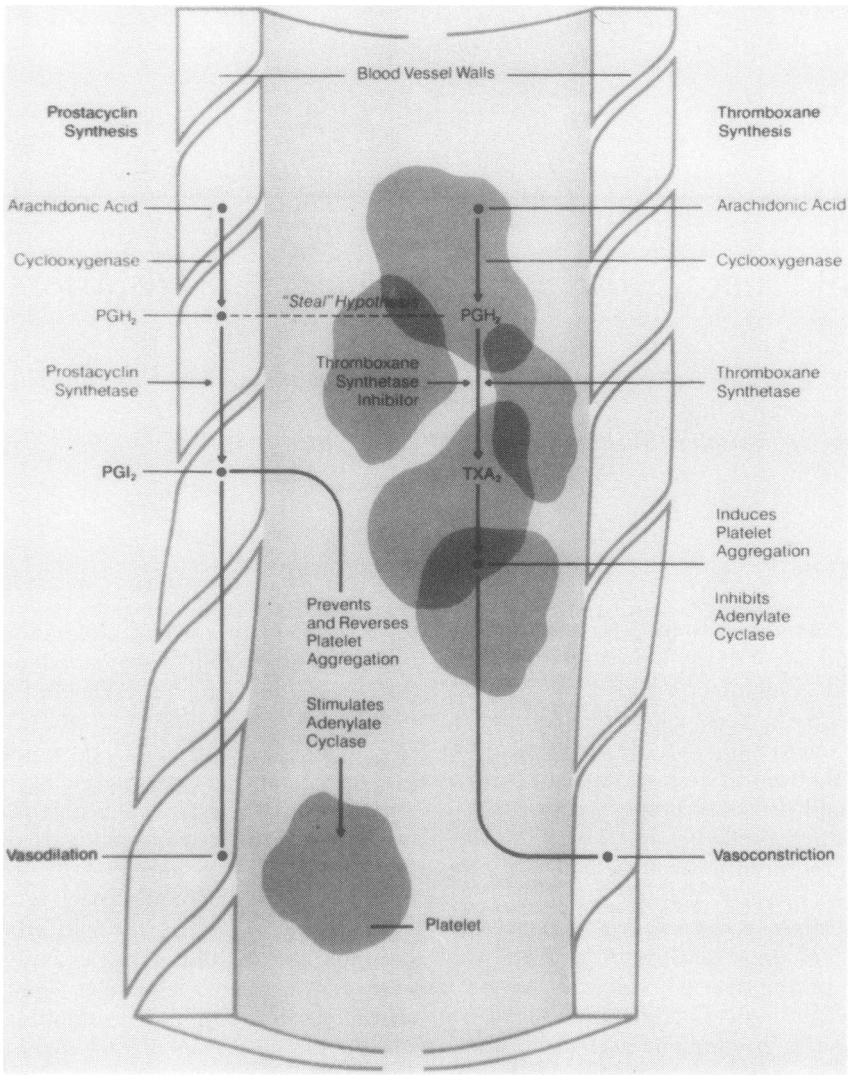

FIGURE 1 Arachidonate metabolism in endothelium and platelets. TXA_2, thromboxane A_2. (From Gorman, R. R., and A. J. Marcus. 1981. Prostaglandins and Cardiovascular Disease. *In* Current Concepts. The Upjohn Co., Kalamazoo, MI)

acts by increasing platelet cyclic AMP levels. High cyclic AMP levels in platelets raise the threshold for response to thrombin and other agonists. PGI_2 action is opposite to that of thromboxane A_2. Moncada and Vane (8) proposed that vascular homeostasis is determined by a balance between the production of thromboxane A_2 and PGI_2 and that the occurrence of thrombotic disorders might be influenced by factors that alter this balance. Thus, the use of aspirin might have paradoxical effects, since inhibition of endothelial cyclooxygenase by aspirin might actually promote thrombosis. Recent studies indicate that this speculation is probably incorrect. I will now address several questions that have been the subject of recent investigations.

What is the source of arachidonate for vascular PGI_2 synthesis? Studies with cultured cells and isolated vessels indicate that endothelial cells have receptors for thrombin (9), bradykinin (10), and histamine (11), and that each of these agonists stimulates arachidonate release from phospholipids with subsequent conversion to cyclic endoperoxides and then to PGI_2. Smooth muscle cells also produce PGI_2 in response to serotonin and platelet-derived growth factor. An alternative source for the substrate for PGI_2 is

cyclic endoperoxides formed in platelets, as outlined in Fig. 1. Endoperoxides may escape from platelets and be converted to PGI_2 by endothelium (8). The phenomenon of "endoperoxide steal" has been demonstrated in vitro (12); its importance in vivo is uncertain, although it may explain the action of thromboxane synthetase inhibitors as outlined below.

Is there a dose of aspirin that blocks thromboxane production without inhibiting PGI_2 production? Early in vitro studies suggested that platelet cyclooxygenase is more sensitive to aspirin than that in vascular cells (13), and that, because of new enzyme synthesis by endothelium, vascular PGI_2 production was restored quickly after aspirin administration (14). However, studies using fragments of human veins incubated in vitro after patients were given aspirin in vivo (15–17) indicated that doses >40 mg of aspirin/d inhibit vascular PGI_2 production for at least 48 h (~60% inhibition of PGI_2 synthesis in response to exogenous arachidonate). In these studies, PGI_2 production was measured in response to the trauma of cutting up the vessel or to exogenous arachidonate. These methods may not assess the ability of vessels to synthesize PGI_2 in response to a physiological agonist in vivo, where arachidonate release may be tightly coupled to cyclooxygenase and PGI synthetase. In another type of study, urinary metabolites of PGI_2 have been measured after aspirin administration. Patrignani et al. (18) have shown that 0.45 mg/kg per d of aspirin inhibited thromboxane production in platelets by >95% without any effect on basal urinary 6-keto prostaglandin $F_{1\alpha}$ levels or on increased levels after furosemide infusion. FitzGerald et al. (19) found that low doses of aspirin (<160 mg/d) totally inhibit urinary excretion of thromboxane metabolites, while PGI_2 metabolites persist at 25–40% of the uninhibited values even with doses of aspirin of 2.6 g/d. These excretion studies are difficult to interpret since it is not certain that the urinary metabolites are derived from vascular sources as opposed to other tissues that synthesize thromboxane A_2 and PGI_2 (e.g., stomach, lung, and kidney). In summary, it appears that platelet cyclooxygenase is more sensitive to aspirin than that in endothelium, but even low doses of aspirin inhibit PGI_2 synthesis in part. High doses of aspirin, however, do not completely block PGI_2 production.

Is inhibition of PGI_2 production associated with any adverse effects? The concept of a balance between PGI_2 and thromboxane A_2 controlling platelet adhesion and thrombus formation implies that PGI_2 is produced by normal endothelium. Several recent studies suggest that this is not the case (20–22). FitzGerald et al. (23) estimate that circulating PGI_2 levels (~3 pg/ml) are ~10-fold lower than the minimal amount that inhibits platelet function. It is, of course, possible that local concentrations of PGI_2 at sites of endothelial injury may reach higher, physiologically important concentrations. Follow-up of patients with rheumatoid arthritis treated with large doses of aspirin does not uncover an increase in thrombosis or atherosclerosis (24, 25). Furthermore, patients with congenital cyclooxygenase deficiency who lack PGI_2 do not suffer from thrombotic episodes (26). A recent study by FitzGerald et al. (27) indicates that patients with atherosclerosis are not PGI_2-deficient but have higher than normal levels of urinary PGI_2 metabolites. Studies of endothelial cells in culture indicate that the nonthrombogenic properties of endothelium are, in fact, not explained by the production of PGI_2 by this tissue (28). An SV40-transformed line of endothelium to which platelets adhere abnormally is not returned to the normal nonadherent state, even with pharmacologic doses of PGI_2. In summary, the importance of PGI_2 in thromboresistance has probably been overemphasized. There is currently no evidence that inhibition of PGI_2 production is an undesirable side effect of pharmacologic inhibition of thromboxane A_2 production.

Does low-dose aspirin (160 mg/d or less) have increased antithrombotic efficacy compared with larger doses? The clinical trials of the efficacy of aspirin in the prevention of strokes and myocardial infarction have all been carried out at doses of greater than or equal to 0.3 g/d (~1 g/d as average). The largest body of data is from six randomized trials of aspirin in men who had survived a myocardial infarction. While none of these trials gives a conclusive result, when all are pooled it appears that aspirin is of modest benefit reducing deaths by 16% and recurrent infarction by 21% (29). It is clear that much lower doses of aspirin will effectively block platelet thromboxane production. In a study of prevention of shunt thrombosis in patients on hemodialysis, 160 mg aspirin per day was shown to be effective (30). Currently, several trials are underway to evalute low-dose aspirin as an antithrombotic agent. In light of the questions about the importance of PGI_2 outlined above, I doubt that increased efficacy will be achieved. It is likely, however, that lower doses of aspirin will be associated with fewer toxic reactions and this is highly desirable, given that the drug is being used as preventive medicine in relatively healthy populations.

Are thromboxane synthetase inhibitors useful antithrombotic agents? Several selective inhibitors of thromboxane synthetase are currently under investigation. Originally it was thought that these would be ideal antithrombotic drugs sparing PGI_2 production while blocking synthesis of thromboxane A_2. However, thromboxane synthetase inhibitors allow accumulation

of cyclic endoperoxides (Fig. 1) in platelets, and the endoperoxides are potent platelet-aggregating agents. In fact, these drugs may act primarily by stimulating production of platelet inhibitory eicosanoids (31). PGI_2 production is increased (27) by endoperoxide steal and conversion of PGH_2 to prostaglandin D_2 (PGD_2) occurs in plasma. Both PGI_2 and PGD_2 stimulate cyclic AMP production in platelets and thereby inhibit platelet function. However, the elevations of PGI_2 and PGD_2 are relatively modest as judged from the levels of metabolites, and the drugs currently known have a very short duration of action. While the final answer will come from clinical trials, it seems unlikely that a drug of this class will surpass aspirin as an antithrombotic agent.

New strategies for altering eicosanoid production

The production of eicosanoids is controlled by cellular mechanisms for uptake and release of arachidonate from phospholipids (32). Elucidation of the mechanism of arachidonate release has proven difficult because a small fraction of cellular arachidonate is liberated in response to agonists. Thus, maximal PGI_2 production results from liberation of only 1% of endothelial arachidonate. In platelets, ~10% of phospholipid arachidonate is liberated in response to thrombin. Arachidonate is liberated from phosphatidylinositol and phosphatidylcholine. Phosphatidylinositol-arachidonate is liberated by a novel pathway involving three enzymes (32); (*a*) a phospholipase C that forms 1,2-diacylglycerol and cyclic inositol phosphate (33), (*b*) a diacylglycerol lipase that forms 2-arachidonoylglycerol (34), and (*c*) a monoglyceride lipase that liberates arachidonate (34). The enzymatic mechanism for release of arachidonate from phosphatidylcholine is not clear, although a phospholipase A_2 enzyme may function in this capacity (35). The main difficulty with invoking a role for phospholipase A_2 is that cells stimulated with agonists liberate mainly arachidonate or other eicosanoid precursors (36) and no phospholipase A_2 has been discovered that has such a fatty acid specificity. While inhibitors of arachidonate-releasing enzymes might be potent pharmacologic agents, no specific inhibitors have been discovered. Drugs such as mepacrine and local anesthetics interact with membrane lipids and interfere with lipase activity, but since they are not enzyme inhibitors but rather bind to substrates, they have no specificity (37). *p*-Bromophenacyl bromide, a potent lacrimator that destroys free SH groups, is also not specific (37). Recently, a diacylglycerol lipase inhibitor (RHC80267) was described (38). Unfortunately, this drug is ineffective in intact platelets even though it does inhibit diglyceride lipase activity in platelet microsomes (Bross, T. E., S. M. Prescott, and P. W. Majerus, unpublished observations). Corticosteroids inhibit arachidonate release in some cells but not in platelets.

Thromboxane and other eicosanoids are all produced from essential polyunsaturated fatty acids that must be provided in the diet. The major source of arachidonate in man is dietary linoleate (18:2, Δ9,12). Many tissues in man lack the desaturase enzymes needed to convert linoleate to arachidonate (20:4 Δ5,8,11,14), and therefore depend on uptake of arachidonate from plasma. Neither platelets (39) nor endothelial cells (40) can convert linoleate to arachidonate. Since arachidonate levels in plasma are low (~1% of free fatty acid), these cells must have an efficient system for uptake and esterification. This is provided by a recently described acyl-coenzyme A (CoA) synthetase that is specific for arachidonate and other eicosanoid precursors (41, 42). Furthermore, the arachidonoyl-CoA synthetase may also be required for normal eicosanoid production in response to agonists. We recently isolated a mutant line of mouse fibrosarcoma cells that lacks arachidonoyl-CoA synthetase (43). This cell line, although relatively deficient in arachidonate, can be repleted by linoleate since it contains desaturase enzymes. When stimulated with bradykinin to produce prostaglandin E_2, the mutant line shows only ~10% of the response of the control line. This suggests that arachidonoyl-CoA synthetase may charge particular phospholipid pools that release arachidonate in response to agonists. Inhibitors of this enzyme might be effective as antithrombotic or even antiinflammatory agents. That changes in the natural history of diseases may be achieved by altering eicosanoid precursor availability is suggested by recent studies in New Zealand black/New Zealand white mice with autoimmune nephritis. These mice were fed diets deficient in arachidonate (44) or diets supplemented with eicosapentaenoic acid (20:5) (45), which competes with arachidonate, resulting in a reduction in active eicosanoid mediators. In both these eicosanoid-deficient states there is an amelioration of the autoimmune nephritis.

In summary, much remains to be learned about the physiologic actions of eicosanoids and of the mechanisms controlling their production. While preliminary results suggest that alteration of eicosanoid production may be beneficial in treating or preventing vascular disorders, the basic information currently available is insufficient to provide definitive plans for intervention.

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