FEATU les

Eicosanoids and the blood vessel wall

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"EICOSANOID S" is the term coined by E. J. Corey to describe the products of the metabolism of the carbon 20 compounds arachidonic acid (5,8,11,14-eicosa- tetranoic acid) and the related polyunsaturated fatty acids eicosatrienoic and eicosapentaenoic acid. The term includes products formed via the cyclooxygenase enzyme complex (prostaglandins and thromboxanes) and via the lipoxygenases (leukotrienes and other monohydroxy and dihydroxy fatty acids). The products of arachidonic acid metabolism differ in various cell types. With rare exceptions, eicosanoids are not stored in cells but are released immediately after biosynthesis. Many eicosanoids are biologically active and act locally in the immediate microenvironment (i.e., they are cellular messengers). Frequently they function to modulate (amplify or inhibit) other biological responses, such as the action of a hormone. The eicosanoids also appear to be physiologic and pathologic mediators in a variety of clinical conditions, e.g., inflammation, immune responses, thrombosis, control of tissue perfusion, and atherosclerotic cardiovascular disease. In recent years, knowledge of the eicosanoids has increased dramatically. Accordingly, this brief discussion will focus on arachidonic acid metabolism by platelets and cells of the blood vessel wall, and includes comments concerning selected situations in which the products of these cells may be involved in the pathogenesis or treatment of cardiovascular disease. For more detailed and comprehensive information the reader is referred to several recent reviews.1-4

Arachidonic acid: sources and metabolic pathways

Arachidonic acid is one of the major unsaturated fatty acids present in the phospholipids of cell membranes; it is derived from the diet and is synthesized in the cells by chain elongation and desaturation of dietary linoleic acid. Arachidonic acid comprises only about 1% of the free fatty acids in plasma (~10 to 15 µM) and circulates bound to plasma albumin; it is also present in the phospholipids and cholesteryl esters of plasma lipoproteins. Arachidonic acid uptake and esterification by cells is brought about by acyl coenzyme A synthetases. Recently, an acyl coenzyme A synthetase specific for arachidonate and other eicosanoid precursors that enhances the uptake of arachidonate from plasma and may be required for normal release of arachidonate in response to agonists has been identified in platelets.5

In response to various stimuli (mechanical, hormonal, and coagulation factors) arachidonic acid is released from cell phospholipids by the action of phospholipases (figure 1). It is subsequently reacylated back into the phospholipids or is metabolized via the cyclooxygenase and/or lipoxygenase pathways to the eicosanoids characteristic of the given type of cell and biological response. (The metabolic pathways that are activated by receptor-mediated stimuli may differ even in a given type of cell.) Phospholipases A, and C (the latter in combination with a diglyceride lipase and monoglyceride lipase) are the calcium-dependent enzymes of the cell membranes that release arachidonate from phosphatidylcholine and phosphatidylserinol, respectively.

The cyclooxygenase enzyme complex oxygenates arachidonic acid to the prostaglandin endoperoxides PGG, and PGH, which are then converted to the various prostaglandins and thromboxanes. The biological activities of these eicosanoids will be discussed subsequently. Recently Samuelsson6 has identified the products of arachidonic acid metabolism by lipoxygenase enzymes in polymorphonuclear leukocytes, macrophages, eosinophils, and mast cells. The 5-lipoxygenase converts arachidonic acid to 5-hydroperoxyeicosatetraenoic acid, which is converted to the corresponding 5-hydroxyacid or to a family of compounds containing a conjugated triene structure, the leukotrienes LTA, LTB, LTC, LTD, and LTE.
FIGURE 1. Pathways of arachidonic acid metabolism.

LTB₄ appears to be important in inflammation; it is a powerful chemoattractant of polymorphonuclear white blood cells and it promotes the transvenular movement of white cells and fluid. LTB₄ also mobilizes calcium in polymorphonuclear leukocytes and induces the discharge of lysosomal contents and superoxide formation. The peptide-containing leukotrienes LTC₄, LTD₄, and LTE₄ collectively account for the biological activity of slow-reacting substance of anaphylaxis.

The metabolites of arachidonic acid in individual cells

Platelets. Platelets contain both cyclooxygenase and 12- and 15-lipoxygenases. The most important platelet cyclooxygenase product is thromboxane A₂ (TXA₂) (figure 1). Thromboxane synthetase in platelets converts PGH₂ to TXA₂, which is a powerful vasoconstrictor that also promotes the activation of platelets and release of the contents of their granules adenosine diphosphate (ADP), serotonin, and platelet derived growth factor. TXA₂ is unstable (half-life of 30 sec in aqueous media at 37°C), but spontaneously forms the more stable thromboxane B₂ (TXB₂). Aspirin covalently acetylates and inactivates the active site of cyclooxygenase in all types of cells. In contrast to other cells, such as those of the endothelium, platelets cannot manufacture new enzyme after aspirin treatment because they are incapable of protein synthesis. Other less active platelet eicosanoids are heptadecatrienoic (HTT), malondialdehyde, and the monohydroxy acids 12- and 15-hydroxyeicosatetraenoic acid (HETE).

Vascular endothelial cells. More than a decade ago, it was discovered that blood vessels synthesize a series of vasodilatory prostaglandins, particularly prostacyclin (PGI₂). In recent years cells of the vessel wall have been studied in tissue culture. Vascular endothelial cells have receptors for angiotensin II, bradykinin, histamine, and thrombin. All of these agonists activate phospholipases and release arachidonate to cyclooxygenase for conversion to the endoperoxides. PGH₂ is converted in endothelial cells to PGI₂ by prostacyclin synthase (figure 1). Recent experimental results indicate that under certain circumstances endoperoxides (PGG₂ and PGH₂) derived from platelets can be utilized by vascular endothelial cells for biosynthesis of PGI₂; this has been called an "endoperoxide steal." Prostacyclin, the major arachidonic acid product of endothelial cells, is a potent vasodilator of the coronary and other vascular beds; it also inhibits platelet aggregation by stimulating adenylyl cyclase and elevating platelet levels of cyclic adenosine monophosphate. Thus its actions are opposite to those of TXA₂. PGI₂ is unstable (half-life of 3 min at 37°C) and converts spontaneously to the more stable 6-keto-PGF₁α.
In liver and kidney, PGI₂ is also metabolized to 6-keto-PGE₁, a metabolite with actions similar to those of the parent compound. Endothelial cells synthesize lesser amounts of PGE₂, also a vasodilator, and PGF₂α, which promotes venoconstriction.

Vascular smooth muscle cells. Vascular smooth muscle cells synthesize the vasodilator prostaglandins PGI₁, PGE₂, and PGF₂α, in response to angiotensin II, serotonin, and platelet-derived growth factor. Prostacyclin production in the vascular smooth muscle cell is less than in endothelial cells, not because of differences in prostacyclin synthase but because endothelial cells contain greater amounts of cyclooxygenase. In 1979 Vane et al. proposed that the balance between TXA₂ synthesis by platelets and PGI₁ synthesis by cells of the vascular wall is important in the maintenance of vascular integrity and the responses of blood vessels to injury and that thrombotic disorders may be influenced by this balance. Although perhaps too simplistic and overemphasized by some, this paradigm has stimulated much basic and clinical research. When endothelial cells are injured and lost from the vessel lining, PGI₁ synthesis is reduced and platelet aggregate and adhere to the site of injury. Possibly in response to platelet-derived growth factor, smooth muscle cells migrate through the internal elastic membrane and form a new lining until the endothelium regenerates. PGI₁ production by these smooth muscle cells increases over time and prevents further platelet aggregation at the site of vascular injury and repair.

White blood cells. Within the lumina, walls, and adventitia of blood vessels may be found polymorphonuclear leukocytes, macrophages, eosinophils, and mast cells, all of which contain lipoxygenases and synthesize monohydroxy and dihydroxy fatty acids and the leukotrienes. 12-Lipoxygenase products of whole blood vessel incubations (12HETE) have been reported, and recently Piper and Vane have found that LTD₄ biological activity was present in a variety of blood vessels, including the pulmonary and coronary arteries. Whether the lipoxygenase products in blood vessels are derived from vascular cells or from white blood cells in the vessel walls is unclear. Similarly, it is not firmly established whether vascular endothelial and/or smooth muscle cells contain lipoxygenases. Preliminary evidence from several laboratories, however, suggests that there may be 12- and 15-lipoxygenase activity in endothelial cells. The lipoxygenase products in vessels are biologically active, in any case. As mentioned previously, LTB₄ is chemotactic and chemokinetic and activates polymorphonuclear leukocytes in addition to inducing movement of white blood cells and fluid across post-capillary venules. LTC₄, LTD₄, and LTE₄ are very powerful constrictors of bronchial smooth muscle (approximately 100 to 1000 times that of histamine), suggesting they may be involved in the pathogenesis of some forms of asthma. The sulfidopeptide leukotrienes in very low concentrations constrict the coronary arteries in a variety of species, are arrhythmogenic, and produce negative inotropic effects. At a microcirculatory level LTC₄, LTD₄, and LTE₄ produce arteriolar constriction and extravasation of fluid and macromolecules across capillaries and venules. Because sustained intracoronary infusions of LTD₄ induce coronary vasoconstriction, followed by a return of coronary flow toward normal, it is possible that the leukotrienes also activate a vasodilatory system in the coronary circulation. In the lung LTB₄ (and also LTC₄) can induce pulmonary vasoconstriction indirectly by activating cyclooxygenase with production of TXA₂.

Eicosanoids and cardiovascular diseases: thrombotic disorders

Although eicosanoids may be involved in a host of illnesses, four types of cardiovascular disease will be mentioned: thrombotic disorders, congestive heart failure, inflammatory disease of the myocardium, and atherosclerosis. The concept that an imbalance between TXA₂ production by platelets and endothelial cell production of PGI₁ might contribute to thrombosis, coronary flow abnormalities due to vasospasm, platelet plugs at sites of coronary obstructions, or transient ischemic events in myocardium and brain has stimulated clinical trials of aspirin in analogous clinical disorders. As summarized elsewhere, six randomized trials of aspirin in men who had survived myocardial infarction were, individually, inconclusive. These studies have been criticized because the large doses of aspirin used for treatment inactivate cyclooxygenase not only in platelets (reducing TXA₂ formation), but also in the vessel walls (reducing PGI₁ synthesis). Nevertheless, the cumulative results (poored) in these 10,893 subjects suggest that aspirin reduced infarction by 21% and deaths by 16%. In two trials in patients with transient cerebral ischemic attacks, aspirin significantly lowered the incidence of stroke or death (42% and 31%), although in one study only men benefitted from treatment. In a recent study in 1266 veterans with unstable angina, aspirin was shown to reduce the rate of infarction or cardiac death in treated patients by 51%. Dipyridamole combined with aspirin also significantly lowered the early and late reocclusion rates of saphenous vein coronary bypass grafts. Use of
aspirin or other drugs to reduce TXA₂ formation in patients with vasospastic angina has been shown to decrease the excretion of the major urinary metabolite of TXA₂, 2,3-dinor-TXB₂; however, it did not significantly reduce the incidence of attacks of chest pain.¹⁰

In an attempt to inhibit TXA₂ synthesis more selectively in thrombotic disorders, the pharmaceutical industry has recently developed drugs that inhibit thromboxane synthase. Administration of one inhibitor, dazoxiben, to normal volunteers was associated with lowered serum TXB₂ levels and increased excretion of the major urinary metabolite of PGI₂ (possibly suggesting endoperoxide steal).¹¹ Since the endoperoxides themselves promote platelet aggregation, however, it is not yet known whether thromboxane-synthase inhibitors will be more beneficial than aspirin as anti-thrombotic agents.

Another strategy to alter TXA₂/PGI₁ balance in patients with thrombotic disease involves dietary manipulation. Results of studies of Greenland Eskimos, whose diet consists largely of fish and who have a low incidence of myocardial infarction, and of normal subjects fed large amounts of cod liver oil or mackerel indicate that the cell membranes in subjects with a high intake of fish oil become enriched in eicosapentaenoic acid at the expense of arachidonate. This eicosanoid also competes with arachidonic acid for cyclooxygenase and/or lipoxygenase and gives rise to prostaglandins and thromboxanes with three instead of two double bonds. TXA₂ has weaker proaggregatory activity than TXA₁. Platelets from fish oil–fed subjects released TXB₂, synthesized less TXB₂, and had decreased aggregability compared with platelets from control subjects. The subjects receiving fish oil also synthesized PGI₁, an eicosanoid that has vasodilatory and antiaggregatory properties similar to those of PGI₂. These results, plus experimental studies showing reduced cerebral and myocardial infarct size in fish oil–fed animals, have spurred worldwide interest in the study of the effects of eicosapentaenoic acid in patients with thrombotic cardiovascular diseases.

Eicosanoids and vascular resistance: congestive heart failure

Local synthesis of vasodilatory prostaglandins by cells of the vessel wall appears to be an important mechanism controlling the level of capillary perfusion in various regional circulations, particularly in the face of vasoconstrictor stimuli such as activation of the sympathetic nerves (norepinephrine), the renin-A angiotensin II system, or vasopressin. In vascular beds such as kidney, the level of vascular tone appears to be determined by the summation of the effects of opposing forces of vasoconstriction and vasodilation. In experimental studies of sodium depletion and acute heart failure, renal perfusion was relatively maintained, despite reductions of cardiac output and increases in total peripheral vascular resistance and significant increases in renal output of renin and norepinephrine.¹²,¹³ Increased renal vascular synthesis of prostaglandins (reflected in renal overflow of PGE₂) in these two situations acted to attenuate the vasoconstrictor influences on renal tone; inhibition of cyclooxygenase induced marked rises in renal vascular resistance and corresponding reductions in renal blood flow. Results of micropuncture studies have extended these observations, indicating that local prostaglandin production during depletion of extracellular fluid volume attenuates the local effects of angiotensin II in the kidney of constriction of efferent arterioles and contraction of glomerular mesangial cells (which lowers the ultrafiltration coefficient of glomerular capillary membranes). It has also been reported that a fraction to produce patients with advanced heart failure have increased plasma concentrations of renin, angiotensin II, 6-keto-PGF₁α, and a metabolite of PGE₂.¹⁴ In these patients the administration of nonsteroidal anti-inflammatory drugs to inhibit cyclooxygenase not only depressed renal blood flow and renal function but increased total peripheral vascular resistance and depressed cardiac output. Additional clinical studies reporting reduced PGE₂ excretion in groups of patients with systemic hypertension and the attenuation of the effects of antihypertensive drug therapy when indomethacin was also administered have raised the possibility that alterations in vascular prostaglandin synthesis may be involved in the pathogenesis of several forms of human hypertension. Although suggestive, clinical and experimental work in this area is currently far from conclusive.

Eicosanoids and myocardial inflammation: infarction, myocarditis, and transplant rejection

Myocardial infarction, myocarditis, and cardiac transplant rejection are three examples of cardiac diseases in which inflammatory cells (polymorphonuclear leukocytes, macrophages, lymphocytes) migrate into the damaged tissue. In all three conditions, the numbers and types of cells differ in different stages of the process of injury and repair. Investigations in other organs (e.g., experimental hydronephrosis) have clearly indicated that the spectrum of eicosanoids produced in the tissues and the consequent physiologic effects vary with the type and number of inflammatory
cells. Preliminary studies from this and other laboratories suggest that synthesis of biologically active cycloxygenase products (e.g., TXA2) and lipoxygenase products (e.g., LTB4, LTC4, LTD4, LTE4) may contribute to the vascular damage, edema, myocardial cell death, arrhythmias, and abnormalities of coronary perfusion and contractility that characterize these disorders.4 In many studies, diverse effects of nonsteroidal anti-inflammatory drugs on the size of myocardial infarctions have been found in experimental animals.15 The discrepancies in these reports probably result from different effects of the individual drugs on the various enzymes involved in arachidonic acid metabolism and also on differences in the cell populations infiltrating myocardial tissue at the time of study. As more selective inhibitors are developed, particularly those specific for enzymes in the lipoxygenase pathway, the influence of specific eicosanoids on the manifestations of cardiac inflammation and infarction should become apparent.

Eicosanoids and atherosclerosis: platelets, PGI2, and high-density lipoprotein

During the past decade, knowledge of the cell biology of atherosclerotic lesions has increased rapidly. The importance of endothelial damage, platelet activation, and macrophage and smooth muscle cell proliferation and migration into the intima where they become foam cells is becoming clearer as a result of various experimental and pathologic studies. Although platelet defects in pigs with von Willibrand disease or administration of aspirin to monkeys fed a high-lipid diet retarded the development of atherosclerotic lesions in these animal preparations, it has been difficult to implicate platelet abnormalities in human atherosclerosis. In one series of patients with angiographically proven coronary artery disease, elevations in levels of fibrinopeptide A, TXB2, or platelet proteins were not detected in peripheral venous blood.16 However, in a recent preliminary report plasma concentrations of TXB2 and urinary excretion rates of 2,3-dinor-6-keto-PGF1α were found to be elevated in a small group of patients with severe peripheral atherosclerosis and platelet activation.17 Results of a significant number of early studies reporting platelet activation in the coronary during or after spontaneous or pacing-induced angina pectoris must be interpreted cautiously because of the woven Dacron and polyethylene cardiac catheters (not heparin-bonded) used for coronary sinus sampling in these studies are known to promote fibrin formation and platelet deposition on their surfaces and artifactually elevate measured values of platelet pro-
teins of TXB2 in the plasma samples. More recently, platelet protein levels in the coronary sinus of patients with coronary disease were found to be normal during pacing-induced angina.*

Studies by Gryglewski et al. and by others2 have indicated that PGI2 synthesis is reduced at sites of early and late experimental and human atherosclerotic lesions. The mechanism responsible for this is unknown. The fact that defective PGI2 formation by smooth muscle cells from atherosclerotic vessels persists with serial passage in tissue culture makes the initial proposal by these investigators that lipid peroxides in lesions inhibit prostacyclin synthase unlikely. The hypothesis that vessel wall lipids induce the production of eicosanoids that inhibit cyclooxygenase is currently under study in several laboratories.

The concentration of plasma high-density lipoproteins correlates negatively with the risk of coronary disease; the mechanism responsible for this beneficial effect in patients with atherosclerotic disease is not known. Recently high-density lipoproteins have been shown to stimulate PGI2 formation by vascular endothelial and smooth muscle cells grown in tissue culture; low-density lipoproteins were also stimulatory in these systems but to a much lesser extent.18,19 In the presence of serum lipids endothelial cells exhibit depressed synthesis of arachidonic acid from linoleic acid. Pomerantz et al. have provided evidence that high-density lipoprotein cholesteryl esters not only supply arachidonate to endothelial cells as substrate for PGI2 synthesis but also enrich the stores of arachidonate in endothelial cell phospholipids.4 Other research has implicated high-density lipoproteins and PGI2, and its metabolites in the removal of cholesterol from cultured smooth muscle cells.20 Further work is required to investigate whether these phenomena that have been demonstrated in vitro are involved in the antiatherogenic effects of high-density lipoproteins in vivo. Another cell type involved in atherogenesis, the macrophage, which becomes a foam cell when exposed to low-density lipoprotein that has been modified by malondialdehyde or endothelial cell–derived culture media, also appears to be an important constituent or atherosclerotic lesions. Macrophages produce a wide variety of cyclooxygenase and lipoxygenase products, including LTB4 and LTC4.4 The role of these cells and of their eicosanoids in the formation of atherosclerotic lesions is also currently under study.

* Nichols AN, Owen J: Unpublished observation.

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expanding family of membrane-derived lipids that exhibit a wide range of biological activities. Synthesized and released by cellular constituents of blood and by endothelial and smooth muscle cells of the vascular wall, these compounds are extremely potent initiators and modulators of biochemical reactions and physiologic responses in the immediate microenvironment. As knowledge of the biochemistry of these products and of their actions increases and as more specific pharmacologic means to inhibit their biosynthesis and target cells responses are developed, the importance of the eicosanoids in the pathogenesis and treatment of cardiovascular diseases will become more apparent.

References
Eicosanoids and the blood vessel wall.
P J Cannon

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