SESSION II: THE EICOSANOIDS: BIOSYNTHESIS, PATHOPHYSIOLOGY, AND PHARMACOLOGY

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Eicosanoids: A Selective Update

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Introduction

The phenomenal and on-going explosion of knowledge regarding the physiological and pathological roles of the eicosanoids, as well as the pharmacological modulation of their biosynthesis and actions, precludes any comprehensive commentary on the current understanding of all aspects of the biosynthesis, pathophysiology and pharmacology of the prostaglandins, leukotrienes and the hydroperoxy fatty acids. Several recent reviews should serve as adequate sources for many of the details of eicosanoid-associated pathophysiology and pharmacology and only a few select topics of special interest will be covered in this presentation. However, a synoptic overview of the arachidonic acid cascades and the main physiological and pharmacological actions of the principle eicosanoids will be furnished to serve as a basis for the succeeding papers in this section. The subjects that will be discussed include the concept of membrane-mediated control of intra- and extracellular events by phospholipid-derived substances, the roles of eicosanoids in membrane ion transport, the renal effects of the prostaglandins and some features of the inflammatory and immune responses that are modulated by the eicosanoids. It is clearly recognized that substantial advances have also taken place with regard to our knowledge of the eicosanoids' actions in many other body systems, such as the gastrointestinal, cardiovascular, reproductive and central nervous systems, but these will not be addressed specifically in this review simply due to the extensiveness of such a task.

Membrane-mediated Control of Cellular Functions

Cellular components that are fundamental and necessary for life, such as the enzymes associated with intermediary metabolism, the cytochrome systems and even the contractile proteins, have changed little through long periods of time and are functionally very similar between various mammalian cells. In contrast to this, substances formed in or around cells in response to an injury or specific stimulus and which initiate characteristic cellular reactions may differ substantially between mammalian species and even between cells in the same animal. Typically, the response to such autacoids are often species and tissue specific. Amongst these agents, the eicosanoids and other phospholipid derivatives have probably evolved into their current complexity relatively recently in the evolutionary time frame and seem to have retained an adaptability to not only species' needs but even to tissue and organ requirements. Tissues characteristically differ in their patterns of prostaglandin synthesis.

Cellular membranes play cardinal roles not only as physical and

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functional barriers but also in initiating appropriate cellular responses to specific physiological stimuli or diverse extracellular perturbations. These mechanisms have been found to be much more complex than was at first realized and are not based solely on the depolarization of an excitable membrane or the passive flux of an appropriate mono- or divalent cation.

In recent years, several distinct systems have been identified through which membrane-mediated activation or inhibition of regulatory molecules may take place. These in turn can modulate specific biochemical events in cells to produce specific tissue responses. Cell membranes are generally composed almost entirely of proteins (about 55 percent), phospholipids (about 25 percent), cholesterol (about 13 percent), other lipids (about 4 percent) and carbohydrates (about 3 percent). It is the protein and phospholipid fractions that are capable of yielding regulatory substances responsible for the maintenance of normal cell function. The roles of membrane proteins in this regard are now quite well known but those of the membrane phospholipids and their derivatives have only been elucidated in recent years.

Briefly, membrane-mediated control of intra- and extracellular events may depend on

A. Membrane proteins
   1) Receptor proteins themselves may form part of an ion channel across the plasma membrane or they may control the entry of ions, such as calcium, into the cell through distinct channels.
   2) Membrane-associated enzymes acting either as protein kinases themselves or by activating protein kinases via cyclic nucleotide mechanisms.

B. Membrane phospholipid derivatives
   1) Through activation of various phospholipases and other enzymes several derivatives may be produced such as
      a) Inositol-triphosphate (Inos-P_3), which releases Ca++ from intracellular stores.
      b) Diacylglycerol (DAG) which, with Inos-P_3, activates a distinct set of protein kinases (protein kinase C) and which also promotes membrane fusion.
      c) Acetyl glyceryl ether phosphorylcholine (AGEPC, PAF or PAF-acether), which increases vascular endothelial permeability and acts as a mediator of inflammation (stimulates neutrophils, macrophages, certain mast cells and platelets).
      d) Several lysophosphatides which are regarded as putative mediators of inflammation at this time.
   2) Through activation of specific phospholipases (PL) unsaturated 20-carbon fatty acids (especially arachidonic acid) are released from membrane phospholipids and are then enzymatically converted to one of a number of classes of eicosanoid such as
      a) Prostaglandins (PGs)

b) Leukotrienes (LTs)
c) Hydroperoxy - and hydroxyeicosatetraneoic acids (HPETEs and HETEs)

The biosynthesis, pathophysiology and pharmacology of these eicosanoids will enjoy the main attention in this review but the fact that several other membrane-derived substances may act either in concert with or antagonistically towards the eicosanoids to modulate cellular responses is an important concept.

Cell Origin of Eicosanoid Mediators

The phospholipids in cells are the source of the major lipid mediators. The most abundant phospholipids in most tissue are phosphotidylcholine (PC), phosphotidylethanolamine (PE) and sphingomyelin (Sph). Phosphotidylserine (PS) and phosphotidylinositol (PI) may also occur in relatively high concentrations in certain cells. Differences between the composition of cells may in fact only involve the relative content of minor components such as lyso-PE (higher in monocytes than polymorphonuclear cells), lyso-PC (relatively abundant in bone marrow macrophages) and lyso (bis) phosphatidic acid (significantly higher concentrations in alveolar macrophages).

The primary eicosanoids may be derived from several 20C polyenoic acids present in phospholipids. The bioconversion of (a) unesterified membrane-associated 8,11,14-eicosatrienoic acid (dihomo-gamma-linolenic acid) leads to the formation of PGE1 and PGF1alpha, (b) 5,8,11,14- eicosatetraneoic acid (arachidonic acid) to the formation of PGE_2, PGF2alpha, PGD2, TXA2 and PGI2, and (c) 5,8,11,14,17-eicosapentaneoic acid to the synthesis of PGE3 and PGF3alpha. In most mammalian tissues, arachidonic acid is quantitatively the most important precursor of the endogenous eicosanoids.

In general, there is considerable diversity in arachidonic acid content and distribution between different cell types and even between different species. Platelets have a very high content of esterified arachidonate. Human polymorphonuclear cells (PMNs) are richer in arachidonate than guinea pig PMNs. Rabbit alveolar macrophages and murine peritoneal macrophages contain three times as much arachidonate as murine bone marrow macrophages. Most often PE is richest in arachidonate followed by PS and PI.

Phospholipids are usually asymmetrically distributed between the two layers of the plasma membrane. Phosphatidylcholine and Sph mostly face the outside whereas arachidonate-rich PE, PS and PI are located in the inner half of the lipid bilayer. This arrangement is compatible with the intracellular release of the major precursor of the prostanoids, leukotrienes and hydroperoxy- and hydroxyeicosatetraneoic acids.

Synoptic Overview of Biosynthesis and Fate of the Eicosanoids
Release of Arachidonic Acid from Phospholipids

Eicosanoids are not stored in cells; their synthesis is initiated by the hydrolysis of fatty acids from cellular phospholipids. This is

normally the rate-limiting step in the formation of eicosanoids due to the low intracellular levels of precursor fatty acids in the cell. The release of arachidonic acid and related eicosapolyenoic acids from membrane phospholipids involves the action of phospholipases (PLs). Activation of these PLs may occur nonspecifically with cell damage or may occur following specific receptor-coupled cell activation.

A. Cellular injury and nonspecific activation of phospholipase A2. Cell or membrane damage is associated with the direct release of arachidonate. Precipitating causes include mechanical trauma, burns, ischemia, cell distortion, inflammation, immunologic reactions, ultra-violet light and cell death. Nonspecific phospholipase A2 (PLA2) activity is more diffusely distributed than the specific agonist-coupled phospholipase activity and results in the release of other fatty acids, such as oleic and linoleic, as well as arachidonic acid. This direct release of eicosapolyenoic acids is poorly coupled to cyclooxygenase enzyme activity.

Cells contain several forms of PLA2 which are distinguished by their pH optima, sensitivity to Ca++ and intracellular localization. The two main activities recognized in many cells are, firstly, those of a lysosomal nature, pH optimum 4.5 and not requiring Ca++ and, secondly, those of a microsomal nature, pH optimum 7.5-8.5 and requiring Ca++. Platelet PLA2 requires calmodulin for activation.

B. Specific agonist-coupled release of arachidonic acid. Four types of phospholipases could potentially be involved in the agonist-mediated release of polyenoic acids from membrane phospholipids. Phospholipase A1 (PLA1), which hydrolyzes saturated fatty acids from the C-1 position, and phospholipase D (PLD), which removes the base from phospholipids to leave phosphatidic acid, are not implicated in making arachidonic acid available for eicosanoid synthesis. PLA2 isozymes are not fatty acid specific (cleaving any fatty acid on C-2) and although PE is the preferred substrate, PC is the phospholipid most often hydrolyzed to yield arachidonic acid. It appears, at least in platelets and perhaps neutrophils, that PLA2 and PC containing arachidonic acid are located at the same specific subcellular site. The second principal pathway for the agonist-coupled release of arachidonic acid from cell phospholipids is initiated by phospholipase C (PLC). PLC hydrolyzes phospholipids to produce diglyceride and the phosphorylated base (e.g. inositol, choline, ethanolamine or serine phosphate). Platelets and other blood cells have PLC that specifically attacks phosphotidylinositols. The immediate product of PLC activity is a diglyceride which is then hydrolyzed by a diglyceride lipase to yield arachidonate and most often stearate. A third possible origin of polyenoic acids is through the action of a phosphatidic acid-specific PLA2 which can remove arachidonic acid from phosphatidic acid.

PLC is often activated first and its activity is closely

coupled to receptor occupancy, e.g. by thrombin in the case of platelets. The reactions involved in the breakdown and resynthesis of phosphatidylinositols illustrate the specific agonist-coupled initiation of PLC activity and subsequent diglyceride lipase activity. PI-specific PLC is a cytosolic enzyme.

Besides the release of arachidonate as a substrate for cyclooxygenase (diglyceride lipase is located in the endoplasmic reticulum, which is also the site of cyclooxygenase, prostacyclin synthetase and thromboxane synthetase activity) the above pathway also facilitates cell activation in a number of other ways, namely,

a. The breakdown of phosphoinositides releases membrane-bound protein.
b. The diacylglycerol (DAG) produced is important for protein kinase C activation.
c. Inos-P3 is an important messenger which causes additional mobilization of Ca++.
d. Phosphatidic acid may be acted on by a specific PLA_2 to form lysophosphatidic acid and additional free arachidonic acid.
e. Lysophosphatidic acid and phosphatidic acid may promote Ca++ flux raising cytoplasmic Ca++ levels; phosphoinositides inhibit this effect.
f. Breakdown of phosphoinositides may release membrane-bound Ca++.

Phospholipase A2 action is probably the major mechanism for the release of arachidonic acid for PG synthesis in platelets and some other cells. PI, PC and PE are the main phospholipid sources. PC is often on the exterior surface of the cell and recent evidence suggests that PLA2 can be released from granules (e.g. neutrophils) upon cell stimulation so that some arachidonic acid may be external.

The liberation of arachidonic acid from cell phospholipids can be inhibited in several ways.

a. Elevated cAMP levels. Agents that raise intracellular cAMP levels (either by activating adenylate cyclase or inhibiting cAMP phosphodiesterase) inhibit the release of arachidonate from platelet phospholipids. cAMP could inhibit PLC in part by restricting Ca++ availability through sequestration in the dense tubular system.
b. Anti-inflammatory corticosteroids. The effect of the anti-inflammatory corticosteroids is mediated through the synthesis of macrocortin and lipomodulin which inhibit the release of arachidonic acid.
c. Calmodulin antagonists. PLA2 seems to require calmodulin for activity and trifluoroperazine, a calmodulin antagonist, inhibits PLA2. However, phenothiazine neuroleptics may also act as direct inhibitors of phospholipase.

d. Inhibitors that bind to PLA2. Examples include bromophenacyl bromide, Wallach and Brown inhibitors, and lipomodulin and macrocortin.
e. Inhibitors that interact with substrate. Examples include mepacrine, chloroquine, chlorpromazine, propranolol, putrescine and spermidine, all of which appear to inhibit PLA2 and PLC.
f. Inhibitors that antagonize calcium action. Ca++ is necessary for both PLA2 and PLC activity. Local anesthetics and indomethacin at high concentrations prevent the binding of Ca++ to the PL enzymes.
g. Serum esterase inhibitors. These drugs have been shown to inhibit platelet PLC.

Synthesis of Eicosanoids

As previously noted, several 20C fatty acids may be metabolized to eicosanoid products. 8,11,14-eicosatrienoic acid (dihomo-γ-linolenic acid) contains three double bonds and is the precursor for PGE1 and PGF1 . Eicosapentaneoic acid is found only in small quantities in most diets but those high in fish are enriched with this fatty acid. It contains five double bonds and is the precursor for the three series prostaglandins, PGE3 PGF3 , PGI3. Eicosapentaenoic acid is converted to cyclooxygenase products less efficiently than is arachidonic acid, but can compete with the enzyme to inhibit production of arachidonic acid derivatives. Arachidonic acid is quantitatively the most important precursor and yields the derivatives containing two double bonds, such as prostaglandin E2 and F2.

Once released into the cell, arachidonic acid may be metabolized by either of two enzyme systems. Cyclooxygenase, present in all cells except mature red blood cells, catalyzes the oxidation of fatty acids to compounds which contain a ring structure. These include prostaglandins, thromboxane and prostacyclin. Lipoxygenase is located primarily in the lungs, platelets and white blood cells, and catalyzes the hydroxylation of the straight-chain fatty acids to leukotrienes and related compounds. Additional enzymes result in the production of specific prostaglandin and leukotriene derivatives. Most of the enzymes that catalyze the conversion of arachidonic acid and other fatty acids to eicosanoids are located in the microsomal portion of the cell.

Synthesis of Prostanoids

Prostaglandin endoperoxide synthetase (also referred to as cyclooxygenase or fatty acid cyclooxygenase) catalyzes the initial step in the formation of prostaglandins from arachidonic acid. It consists of two subunits, each weighing about 70,000 daltons and containing a heme moiety. The enzyme can be separated into two proteins, each with unique enzymatic activity. The first enzyme catalyzes the cyclization of arachidonic acid to prostanoic acid which results in the formation of the unstable endoperoxide intermediate, PGG2. This enzyme is also referred to as cyclooxygenase and has an absolute requirement for heme. The second enzyme, peroxidase, is probably an intrinsic portion of the cyclooxygenase and catalyzes the reduction of PGG2 to a second unstable endoperoxide, PGH2. It is the

cyclooxygenase portion of the enzyme that is inhibited by nonsteroidal anti-inflammatory drugs.

Prostaglandin endoperoxide synthetase expresses unusual kinetics. An initial lag phase is followed by an accelerating phase and then a phase that decelerates until no reaction occurs. The initial phase appears to be due to insufficient peroxide necessary to maintain the oxidized enzymatically active state of heme. As more peroxide intermediate PGG2 is formed, the reaction accelerates. The deceleration phase may reflect the formation of an active radical as PGG2 progressively undergoes peroxidation to PGH2. The selfdestructive nature of the enzyme might serve as a protective mechanism since it should prevent the accumulation of prostaglandin products particularly during inflammatory processes.

The endoperoxide intermediates resulting from cyclooxygenase activity undergo chemical decomposition to a mixture of prostaglandins, malondialdehyde and a 17-carbon 12-hydroxy-heptadecatrienoic acid (HHT) compound. Various enzymes catalyze the formation of specific prostaglandins from PGH2.

Prostaglandin endoperoxide D or E isomerases catalyze the formation of the respective beta-hydroxy ketone, PGD2 or PGE2, from PGH2. The D isomerase enzyme is located in the cytoplasm, rather than the microsomal fraction of the cell. PGD2 may also be formed from the non-enzymatic breakdown of either PGG2 or PGH2. Prostaglandins of the D series occur naturally and have potent biological effects. Prostaglandins A2, B2 and C2 result from the dehydration and isomerization of PGE2 and differ only in the position of a double bond located in the ring structure. PGA2 may be formed from PGE2 in vitro by acid treatment and PGB2 from PGE2 following treatment with alkali. Although enzymes capable of interconverting prostaglandins A, B and C have been described in plasma, they probably are not important physiologically. An isomerase catalyzing the conversion of PGC from PGA has been identified in the plasma of several species.

PGF2alpha is formed by reduction of PGH2. The enzymatic nature of reduction remains controversial although the appropriate enzymes have been identified. Uterine microsites of several species contain a unique, potent, PG endoperoxide Falpha reductase that is not present in other organs. PGF2alpha formation can also be catalyzed by a 9-ketoreductase which is not present in the uterus. PGF2alpha reductase enzyme activity has not been detected in the lung, liver or other organs of some species despite the fact that these organs may form relatively large amounts of PGF2alpha.

PGH2 is also metabolized into two unstable products with marked biological activity, prostacyclin (PGI2) and thromboxane (TXA2). Prostacyclin synthetase catalyzes the conversion of PGH2 to PGI2. Like cyclooxygenase, this enzyme also contains a heme moiety. Thromboxane synthetase converts PGH2 to TXB2. TXA2 is an extremely labile intermediate between PGH2 and TXB2.

Most estimates of the amount and type of prostanoid content in

various organs result from experiments which failed to prevent endogenous prostanoid biosynthesis during tissue preparation. "Basal" levels measured by these methods are not very informative. Methods that estimate the capacity of an organ to synthesize prostanoids are probably more useful. However, support for a physiological role for a prostanoid in an organ requires not only evidence of biosynthesis but also the demonstration that natural or hormonal factors stimulate its release from that organ.

Synthesis of Leukotrienes

Lipoxygenases form peroxides of arachidonic acid at various positions to produce the corresponding hydroxy or hydroperoxy fatty acids. The primary products are hydroperoxyeicosatetraenoic acids (HPETEs) which can be further metabolized to leukotrienes (LTs).

The term leukotrienes reflects the original discovery of these compounds in leukocytes. Leukotrienes have a triene structure (three conjugated XC-C double bonds) and a peptide chain linked via a thiol bond to C5. Unlike the true prostaglandins, leukotrienes do not have a cyclopentane ring. Five series of naturally occurring leukotrienes (LTs) have been identified. Each represents a sequential step in the synthesis and is designated by a letter of the alphabet: A, B, C, D and E. As with prostanoids, the number following the letter refers to the number of double bonds in the structure. The most abundant naturally occurring LTs contain four double bonds.

The reaction kinetics of the lipoxygenases are similar to those of prostaglandin endoperoxide synthetase: an initial product-induced (peroxide) activation is followed by a later product-induced inactivation. A number of lipoxygenases have been discovered, each catalyzing the oxidation of arachidonic acid to the corresponding HPETE. The first discovered was 12-lipoxygenase in lungs and platelets. The most important lipoxygenase is 5-lipoxygenase which converts arachidonic acid to 5-HPETE.

5-HPETE represents a branch in the synthesis of leukotrienes and related products. It can be deoxygenated to monohydroxyeicosatetraenoic acid (5-HETE) or dehydrated to the 5,6 epoxide, LTA4. Both 5-HPETE and 5-HETE have biological activity. LTA4 may be hydrolyzed to LTB4 or transformed via glutathione-S-transferase to the glutathione derivative LTC4. The removal of glutamic acid from LTC4 by gamma-glutamyl transferase yields LTD4 and cleavage of glycine by cysteinylglycinase yields LTE4. The formation of LTF4 by the reincorporation of glutamic acid is the final step in leukotriene synthesis. Metabolites of 12- and 15-lipoxygenase activity are also probably biologically significant and are currently under investigation.

Inhibitors of Eicosanoid Synthesis

Inhibitors of Prostanoid Synthesis

Cyclooxygenase inhibitors are of three general types.

1. Reversible competitive inhibitors which include fatty acids such as eicosapentaenoic acid at low concentrations, and oleic, linoleic and linolenic acids at higher concentrations. Ibuprofen, mefenamic acid and BL-365 are effective reversible

inhibitors probably due to attachment to the active heme component in the enzyme. Salicylate and indomethacin are also reversible inhibitors.

2. Irreversible inhibitors of cyclooxygenase include aspirin, fluriprofen and meclofenamic acid. Aspirin acts by acetylating serine at the active site of the enzyme.
3. Acetaminophen is an example of the inhibitors that have antioxidant or radical-trapping properties. Acetaminophen is effective only in tissues which have low endogenous levels of peroxide and not in cells with high peroxide levels, such as platelets.

The sensitivity of cyclooxygenase to non-steroidal antiinflammatory drugs (NSAID) such as ibuprofen, mefenamic acid and aspirin parallels the potency of these drugs. The sensitivity varies between tissues presumably due to subtle differences in enzyme structure. Drugs that specifically inhibit cyclooxygenase increase the concentration of arachidonic acid available to lipoxygenase that may be present in the same cell, thus increasing the production of leukotriene and related products. Hypoxic conditions may also shift the metabolism of arachidonic acid from the cyclooxygenase to the lipoxygenase pathway.

In addition to the exogenous compounds which inhibit cyclooxygenase, there are endogenous protein factors in the plasma of some mammalian species that are capable of inhibiting cyclooxygenase activity while enhancing the enzymes associated with prostaglandin metabolism (15-PGDH, see later discussion). Factors that activate prostacyclin synthetase and inhibit lipoxygenase activity have also been identified in human serum. These factors have not been well characterized.

Alkyl hydroperoxides inhibit prostacyclin synthetase probably by modifying the heme component of the enzyme. Tranylcypramine also inhibits prostacyclin synthetase but has other effects in cells and is not selective for its antiprostacyclin activity.

Compounds which are selective inhibitors of thromboxane synthetase include imidazole, OKY-1581 (a substituted pyrridine), and the imidazole derivatives, dazoxiben and UK-38,485. Some agents inhibit both thromboxane synthetase and cyclooxygenase (e.g., sulfasalazine).

Inhibitors of Leukotriene Synthesis

Many of the lipoxygenase inhibitors are nonspecific in their action and have other effects on cells. There are a number of agents that specifically inhibit lipoxygenase. Acetylenic analogues of arachidonic acid contain triple (rather than double) bonds which prevent dehydrogenation and peroxidation of arachidonic acid. These compounds are competitive inhibitors of lipoxygenase. Other specific inhibitors include: 15-HETE, an inhibitor of 5-lipoxygenase; naturally occurring retinoids which act as antioxidants and selectively inhibit 5-lipoxygenases; escultin which inhibits platelet 12-lipoxygenase and basophil 5-lipoxygenase; low doses of

3,7,10,13-eicosatetraynoic acid (ETA); and 5,8,11,14 ETA which covalently binds and inactivates the active site of 5-lipoxygenase. 5-lipoxygenase is also inhibited by a series of naturally occurring inhibitors such as caffeic acid and synthetic drugs such as nafazatrom.

Some compounds inhibit the formation of selective leukotrienes. Diethylcarbamazine inhibits LTC4 and LTD4 synthesis in the mast cells of some species, probably by blocking the conversion of 5-HPETE to LTA4. Salazosulfapyridine (sulfasalazine) may inhibit the enzyme responsible for the conversion of LTA4 to LTC4. LTC4 is also inhibited by pirpipost, a compound which inhibits 5-lipoxygenase activity. Gamma-glutamyl transferase can inhibited by d-glutamyl phenylhydrazine and serin-borate and these may in turn prevent the formation of LTD4 from LTC4. L-cystein will reversibly inhibit the enzyme that catalyzes the formation of LTE4 from LTD4.

Inhibitors of Cyclooxygenase and Lipoxygenase

Compounds that inhibit both cyclooxygenase and lipoxygenase ("dual blockers") include the following: lipases, 5,8,11,14- and (at high doses) 3,7,10,13-eicosatetraynoic acid (ETA); phenidone and nordihydroguiaretic acid (NDGA) which inhibit 5- and 12-lipoxygenase and cyclooxygenase; BW755C, a pyrazoline analogue of phenidone; and benoxaprofen. BW755C prevents the peroxidation of arachidonic acid.

Degradation and Fate of Eicosanoids

Degradation of Prostanoids

The enzymes responsible for the degradation of locally produced prostanoids are distributed throughout the body. The lungs are the major site for the inactivation of circulating prostanoids, except PGI2. The degradation of prostanoids is very efficient and the halflives of most are very short. The half-life of TXA2 is 30 seconds and that of the others is less than 5 minutes. Since the natural production of prostaglandins of the A, B and C series does not appear to be significant, their catabolism will not be discussed.

The initial step of metabolism of prostaglandins is uptake from the extracellular space into the cytoplasm of the cell containing degradative enzymes. Although prostanoids are lipids, they do not pass freely across biological membranes but are transported by a poorly understood carrier mechanism. Uptake of the E, F and D series prostaglandins by the lungs appears to be stereospecific and requires energy. Little or no uptake of PG metabolites or PGI occurs in the lungs. Uptake can be blocked by high concentrations of NSAIDs.

The profile of plasma and urinary metabolites of prostaglandins is very complex and has been extensively studied. Degradation of prostaglandins can be one of two types: an initial rapid step catalyzed by prostaglandin specific enzymes and a second slower step catalyzed by enzymes similar to those responsible for the ω- and Beta-oxidation of most fatty acids. The remaining discussion regarding the degradation of prostaglandins will focus on the initial, prostaglandin-specific step.

The enzyme that catalyzes the degradation of the classical prostaglandins, 15-hydroxyprostaglandin dehydrogenase (15-PGDH), is located in the soluble portion of organ homogenates. Two types of 15PGDH have been identified. Type I is located in most organs and requires NAD+ for activity. Type II is found in the kidney, brain and erythrocytes and utilizes NADP+ as a cofactor. The best substrate for both types is PGE2 and to a lesser degree, PGF2 . The net effect of 15-PGDH activity is inactivation since the metabolites have little biological activity. There are at least two exceptions to this generalization. A PGF2 metabolite can induce bronchoconstriction similar to the parent compound. In addition, w-hydroxylase is an enzyme located in the cytochrome P450 system of various organs of some species. This enzyme is responsible for the conversion of PGE2 and PGF2 to hydroxyprostaglandins, which retain some biological activity. In addition to the specific catabolic enzymes discussed, enzymes located in the liver, kidney and intestine will nonspecifically degrade several fatty acids, including prostaglandins.

Prostaglandins of the E, F and D series may be enzymatically interconverted. PGEs are reduced by prostaglandin 9-ketoreductase to the corresponding PGF compound. Large amounts of this enzyme are found in the kidney and parts of the gastrointestinal tract. The reverse action may also occur: PGF compounds may be converted to PGEs by prostaglandin 9-hydroxydehydrogenase (9-HDH). The differences between the actions of PGE and PGF are marked and these two enzymes may thus be important for the regulation of prostaglandin actions in some organs, such as the kidney. PGD2 is also converted to PGF2 by prostaglandin 11-ketoreductase. Although this enzyme has been purified from animals, its physiological relevance is not known.

Prostacyclin spontaneously hydrolyses to 6-oxo-PGF1alpha. Prostacyclin may also be enzymatically catabolized as it is a good substrate for 15-PGDH. The PGI2 derivative produced may be further catabolized to PGE2 and PGF2 . A second major enzymatic pathway for PGI2 is oxidation by prostaglandin -9HDH to 6-oxo-PGE1. This enzyme is particularly abundant in platelets. Unlike the other metabolites of PGI2, 6-oxo-PGE1 retains considerable biological activity.

TxA2 also undergoes spontaneous hydrolysis to TxB2, the major route of catabolism in the body. TxB2 may also be catabolized by 15PGDH and other enzymes.

Inhibitors of Prostanoid Catabolism

Several drugs that inhibit 15-PGDH result in the accumulation of the classical prostaglandins. Examples include sulfasalazine and its analogues, carbenoxolone, diphloretin phosphate, indomethacin and fluriprofen. The anti-ulcer effects described for some of these drugs may be due to their inhibition of 15-PGDH.

Mechanisms of Eicosanoid Action

Prostanoid Receptors

Prostaglandins and related compounds will promote cellular activation in some cells whilst inhibiting it in others. In addition,

different prostaglandins may have opposing effects in the same cell. The events leading to these processes are complex.

Prostanoids, like most other biologically active substances, exert their effects by combining with selective prostanoid receptors on cell membranes. Evidence for prostanoid receptors is provided by studies utilizing radioligand binding techniques and by the characterization of biological actions caused by prostanoids and their analogues.

Several classification systems have been proposed for prostanoid receptors. These include classification according to the binding capacity in organ homogenates versus membranes and the prostanoid to which the receptor is most sensitive. A receptor classification system has also been generated from various studies based upon the rank orders of agonist potency expressed by the natural prostanoids. The majority of tissues do not display characteristic rank orders of potency.

Receptors studies utilizing prostanoid agonists and the selective antagonists SC-19220 and AH19437 in platelets have led to the proposal F2, I2 AND TXA2. Similar studies further suggest that at least two prostanoid receptors, PGE2 and TXA2 can be further subdivided. Separate receptors may also exist for PGE1. Receptors for various prostaglandins have been identified in several organs (Tables 1 and 2).

Table 1. Major prostanoids synthesized in mammalian organs.a

Tissue	Prostanoid	Comments
Lung	6-oxo-PGF1α > TXB2 > PGE2 > PGF2	
Kidney	PGE2- >PGF	TXB2 in ureter obstructed kidney
Spleen	TXB2 > 6-oxo-PGF1α > PGE2	
Platelets	TXB2 > PGD2, PGE2 > PGF2	
Uterus	6-oxo-PGF1α > PGE2 > PGF2	
Corpus luteum	6-oxo-PGF1α > PGE2	
Stomach	6-oxo-PGF1α > PGE2 > PGF1	Very species dependent
Colon	6-oxo-PGF1α = PGD2 = PGE2	Very species dependent
Brain	PGD2 > TXB2	
Blood vessels	6-oxo-PGF1α	PGE2 in placental vessels
Adipocytes	6-oxo-PGF1α > PGE2 = PGF2α	
Gall bladder	6-oxo-PGF1α >> PGE2 = PGF2α	

(a) Moore PK, 1985

Table 2. Organs in which prostanoid receptors have been identified.(b)

PGI2	Human: platelets, uterus, corpus luteum Cow: coronary artery Guinea pig: pulmonary artery
PGE1/PGE2	Human: platelets, uterus, corpus luteum, sperm Cow: platelets, pineal gland

	Guinea pig: uterus, corpus luteum, skeletal muscle Rat: platelets, uterus, adipocytes, kidney, thymocytes stomach, adrenal gland, fibroblasts, liver, skin, neuroblastomal cells
PGF2 α	Human: uterus, corpus luteum, sperm Hamster: uterus Rabbit: oviduct Rat: corpus luteum Horse: corpus luteum Cow: corpus luteum
PGD2	Humans: platelets
PGA2	Rabbit: kidney

(b) Moore, PK, 1985

Intracellular Mechanisms

There are at least two and probably four mechanisms by which cells may be activated. These include calcium flux mediated events, activation of protein kinase C, which interacts with calcium, cell-cell adhesion and cAMP-mediated cell stimulation. Two mechanisms by which prostanoids may mediate their effects include the induction of calcium fluxes and direct interaction with cellular proteins. The two mechanisms are not mutually exclusive.

A considerable amount of evidence links the activity mediated by prostanoid derivatives of arachidonic acid (in platelets) to initiation of calcium fluxes necessary for cellular activation. For example, TXA2-induced platelet aggregation is mediated by increased levels of intracellular calcium which in turn stimulates the interaction of actin and myosin. Although PGG2, PGH2 and TXA2 may increase intracellular calcium by acting as calcium ionophores, their actions are probably more complex. Other proposed mechanisms of mediating calcium fluxes have been studied. The roles of cAMP and adenylate cyclase in calcium flux are discussed below. PGE increases uterine intracellular calcium but apparently does not inhibit adenylate cyclase.

Cellular activation by products of lipoxygenase also appear to involve modulation of calcium flux. Both hydroxy fatty acids and leukotrienes appear to activate neutrophils by promoting calcium flux. LTB4 appears to act as a calcium ionophore.

PGG2 lowers the calcium requirement necessary for the enzymatic production of cyclic guanine monophosphate (cGMP) in platelets, suggesting that TXA2 may mediate its effect by raising cGMP levels. This, however, is not the activating factor in platelets and may simply provide a local energy source. Other studies suggest that while lipoxygenase and cyclooxygenase metabolites can activate cGMP in cells, they are minimally effective. The relationship between cGMP activation and eicosanoids is probably variable and tissue specific.

Many of the effects of prostanoids are mediated by changes in

intracellular levels of cyclic 3'5' adenosine monophosphate (cAMP). Prostaglandin endoperoxide analogues can increase protein phosphorylation, suggesting that PGG2/PGH2 and TXA2 may produce their effects by interacting with enzymes that promote phosphorylation. The inhibitory effect of cAMP appears to result from activation of protein kinases and phosphorylation of cell proteins. Calcium sequestration may be the mechanism by which cAMP mediates this effect. For example, inhibition of platelet aggregation by prostacyclin is mediated by increasing cAMP and calcium sequestration.

In many tissues, prostaglandins regulate the synthesis of cAMP by adenylate cyclase activation or inhibition through mechanisms similar to that for other hormones. For example, in the adrenal glands, PGE stimulates steroid formation by activating adenylate cyclase, but stimulates epinephrine induced lipolysis by inhibiting adenylate cyclase. Prostanoid receptors in at least some cells (platelets and corpus luteum) are linked to a membrane adenylate cyclase enzyme. PGE1 has been shown to produce elevated platelet cAMP by interacting with adenylate cyclase; the enzyme itself being the PGE1 receptor. Recent studies suggest that two types of prostanoid receptors are linked to adenylate cyclase in platelets: one which interacts selectively with PGD2 and another which interacts with PGI2 and PGE1. These different receptors may show different affinities for each PG. For example, studies suggest that there are two PGI receptors, one with a high affinity that will also interact with PGE and one set with a lower affinity that interacts with PGD. This may explain why relatively high concentrations of PGE will mediate effects similar to PGI in platelets.

Modulation of cAMP levels by eicosanoids probably involves mechanisms other than modulation of adenylate cyclase. For example, cAMP phosphodiesterase may be directly inhibited.

Receptor Antagonists

While there presently are not effective receptor antagonists for PGs or LTs, several compounds are effective in vitro and eventually may prove useful in vivo. Three types of prostaglandin antagonists are important: the 7-oxa analogues of prostanoic acid, dibenzoxazepine hydrazine derivatives (eg, SC-119220) and polyphloretins, polyanionic polyesters of phloretin and phosphoric acid.

Some compounds appear to be specific receptor antagonists. The inhibitory effects of endoperoxides on TXA2- presumably is due to interaction with receptors. Prostanoids with a bicycloheptane ring and 13-azaprostanoic acid will also antagonize TXA2 receptors. Chromone carboxylic acid is a potent and specific antagonist of SRS-A. However, its biological half-life is too short for clinical application. A number of LTD4 receptor antagonists have been described, including some promising orally active drugs.

HPETEs and HETEs

HPETEs and HETEs both have significant biological activity. The majority of research has focused on their ability to modulate white blood cell activity. In neutrophils, HETEs stimulate chemotaxis,

although less so than LTB4, and 5-HETE potentiates granule secretion mediated by LTB4 and other modulators. 15-HETE is produced by T-lymphocytes and progressively inhibits their mitogenic response to T-cells. In contrast, 5-HETE enhances migration of human T-lymphocytes. While 15-HPETE appears to be important in the activation of monocytes, the principal lipoxygenase metabolite of human monocytes is 5-HETE.

The addition of 5-HPETE and 5-HETE to human basophils will enhance antigen-induced histamine release, whereas the addition of other lipoxygenase products has no effect. Similar findings in other species suggest that 5-HPETE and 5-HETE are the active products in basophils.

Lipoxygenase products can be produced in endothelial cells and recent studies show that they may be important in modulating migration. For example, 15-HETE stimulates endothelial cell migration while 12-HETE reduces migration.

Selected Effects of Eicosanoids on Membrane-ion Transport

The study of the interactions between ion transport and eicosanoids is difficult because the proteins involved are integral parts of the cell membrane and thus are almost impossible to extract and purify. The ability of PGs to mediated epithelial ion transport has been recognized for some time. PGs can stimulate electrogenic anion secretion by the mucosal epithelium of the gastrointestinal tract. Because they are associated with inflammatory lesions, PGs are probably important mediators in a variety of intestinal disorders, such as secretory diarrhea. LTs and related products are currently being investigated for their role in modulating intestinal secretions. Early studies show differences in the effects of the various lipoxygenase products. These effects differ for each region of the gastrointestinal tract studied. Both 5-HPETE and 5-HETE stimulate anion secretion in the colon, whereas other lipoxygenase products are not effective. The effects of 5-HPETE and 5-HETE do not seem to be mediated by cyclic AMP or GMP.

The effects of PGs of the E series on renal tubular ion transport are potent. PGE inhibits active transport of NaCl as well as electrogenic Na+ absorption. PGE also inhibits vasopressin mediated Na+ absorption in the collecting tubules. PGF2 acts similarly to PGE in this region. Since the mechanism of Na+ reabsorption differs in each region, the mechanisms by which PGE inhibits ion transport in the kidney are probably complex and currently under investigation.

Eicosanoids may also be involved in ion transport regulation of non-epithelial cells by activating adenylate cyclase. Studies that measure the flux of Na+ and K+ across cell membranes have yielded some information regarding the role of PGs and LTs in ion fluxes in nonepithelial cells (e.g. red blood cells). All PGs, but particularly those of the E and I series, appear to inhibit the Na+/K+ cotransport system and stimulate the Na+/K+ pump and the Na+/Ca++ exchange mechanism. Since similar changes occur following the administration of beta adrenergic antagonists, these studies suggest

that the ion transport modulation by PGs is mediated by cyclic AMP. PGE_2 causes elevated cyclic AMP levels probably by activating adenylate cyclase. Cyclic AMP, in turn, stimulates a chloride transport in several systems (e.g., frog cornea). However, chloride transport increased in studies using indomethacin to block the formation of PGE2, suggesting that other metabolites of arachidonic acid also played a role in chloride transport. Both LTB4 and LTC4 are potent mediators of chloride transport. The mechanisms for LT modulation of chloride transport is not known.

Selected Effects of Eicosanoids and Renal Function

The effects of prostaglandins on the kidneys warrant special attention. These organs have a great capacity for prostaglandin synthesis; stimulants of prostaglandin release include ischemia, calcium, hypertonic glucose, mannitol, sodium chloride, angiotension II, bradykinin, norepinephrine, and mechanical trauma. All types of prostaglandins are formed in the kidneys, but PGE_2 predominates. Although production varies considerably among sites within the kidneys, the bulk of the synthesis occurs in the medulla. Prostaglandins are intimately involved in the physiologic regulation of renal circulation, particularly glomerular filtration.

Prostaglandins influence renal blood flow by modulating afferent and efferent arteriolar tone. PGI2 probably is responsible for the maintenance of normal renal vascular tone. Infusions of PGI2 and PGE2 reduce renal resistance and increase blood flow; these agents also cause renal vasoconstriction, probably because they can augment renin release. TXA2, a potent vasoconstrictor, might contribute to the intense vasoconstriction that accompanies some renal disturbances, for example, ureteral obstruction. The fundamental renal action of most prostaglandins is local attenuation of neural- or hormonal-mediated (e.g., norepinephrine or angiotensin II) renal vasoconstriction. Renal vasoconstriction stimulates prostaglandin synthesis, which in turn results in vasodilation; the deleterious effects of prolonged renal vasoconstriction are thus prevented without affecting other vascular beds. Prostaglandins apparently can redirect renal blood flow from the superficial cortex to the inner cortex. They might mediate the effects of some vasoactive drugs that alter blood flow, for example, furosemide.

The effects of prostaglandins on the glomerular filtration rate usually parallel their effects on renal blood flow and reflect the status of the renin-angiotensin system. The maintenance of compensated kidney function in chronic renal disease might depend on endogenous prostaglandin production. The use of a NSAID might cause acute deterioration of the glomerular filtration rate in patients with chronic renal disease.

Prostaglandins also play an important role in mediating renin release during renal hypotension. PGE2 and PGI2 increase plasma renin activity, and it is likely that PGF2 inhibits renin release. The mechanism of renin regulation is probably mediated through cAMP. Prostaglandins apparently modify the activity of renin after it is secreted by the kidney. PGE2 suppresses the generation of angiotensin

I in the plasma and inhibits the pulmonary conversion of angiotensin I to angiotensin II. Angiotensin II might exert its negative feedback effect on renin release by impairing renal prostaglandin stimulation.

Electrolyte transport in the kidneys is altered by prostaglandins according to several mechanisms. Prostaglandins counterbalance angiotensin II-induced vasoconstriction of the efferent arteriole, thus increasing the glomerular filtration rate and shifting the balance of Starling forces within the peritubular capillary away from proximal tubular reabsorption. This inhibition of proximal tubular reabsorption is probably another major function of renal prostaglandins, which also help ensure the delivery of a sufficient quantity of filtrate beyond the proximal tubule during conditions (e.g., renal ischemia or hypovolemia) in which filtration and distal delivery are threatened.

PGE and other prostaglandins mediate changes in passive Na+ reabsorption due to their side effects on medullary tonicity. Blood flow rate is a major determinant of medullary tonicity; increasing blood flow to the inner cortex increases flow through the efferent arterioles and thus to the medulla. Consequently, Na+ reabsorption and medullary tonicity decrease. Passive reabsorption of Na+ is also decreased by the inhibitory effect of prostaglandins on Cl-reabsorption in the ascending thick loop of Henle. They also can directly inhibit Na+ reabsorption in the distal nephron.

The renal concentrating mechanism is also susceptible to the effects of prostaglandins. The formation of concentrated urine requires inner medullary hypertonicity and maximal water permeability of the collecting duct. Prostaglandins reduce urea accumulation in the medullary interstitium by altering the effects of vasopressin (ADH) on the collecting ducts. Poor urea reabsorption, coupled with the decrease in Na+ reabsorption after increased medullary blood flow, results in a decreased medullary interstitial osmolarity. A more dilute urine will be excreted. Prostaglandins affect water balance by virtue of their natriuretic effects and possibly by a PGE controlled blockage of ADH-induced water reabsorption.

PGE induces erythropoiesis by stimulating the release of erythropoietin from the renal cortex. It has variable effects on red blood cell fragility; low concentrations tend to decrease fragility, but high concentrations increase it.

Selected Effects of Eicosanoids on Inflammation and the Immune Response

PGE2 and PGI2 contribute to the cardinal signs of inflammation, primarily because they cause vasodilation and increase vascular permeability. Prostaglandins increase circulation, heat, redness, and swelling in affected areas; they also can induce hyperalgesia or pain directly or by augmenting the effects of other substances, for example, histamine or bradykinin. Inflammatory cells are drawn to areas of injury by the chemotactic properties of PGs, LTs and HETEs; loss of function results. These cells serve as additional sources of PGs and LTs; macrophages, mast cells, and basophils generate and

release prostaglandins when properly stimulated. Their effects on platelets help initiate inflammation.

Leukotrienes, resulting from lipoxygenase action, also are released from white blood cells but they have greater chemotactic, humoral, and cellular activity than prostaglandins. Some symptoms of rheumatoid arthritis probably result from prostaglandin inflammatory activity since the rheumatoid synovium produces increased amounts of PGE2 and PGF2 . PGE2 and PGF2 in iris tissue might cause increased intraocular pressure and aqueous protein production during ocular inflammation.

Prostaglandins are also involved in anti-inflammatory activity. PGE reduces the release of lysosomes from neutrophils and mediators of inflammation from mast cells. The ratio of PGE2 and PGF2 might inhibit vascular dilation and the increased permeability caused by PGE2. Monocytes and macrophages might modulate their differentiation function and might regulate other cells, for example lymphocytes, through prostaglandins.

Cellular and humoral immune responses are negatively controlled by prostaglandins. PGE2 inhibits T-cell proliferation, lymphokine production, and the generation of cytotoxic cells, decreases humoral antibody production, and might regulate B-cell function. Prostaglandins are necessary but not sufficient for the inhibition of humoral immunity. Various macrophage functions are also regulated by prostaglandins.

Summary Overview of Some Recognized Functions of the Eicosanoids

Prostaglandins (PGs)

1. Reproductive processes
   a. Myometrium - strongly contracted by PGF2α , PGE's effects variable.
   b. Corpus luteum - luteolytic (PGF2alpha). PGE2 also luteolytic.
   c. Semen - transport of sperm in male and female genital tracts.
   d. Menstrual fluid - spasms (PGE2 and PGF2α).
   e. Parturition - PGE1, PGE2, PGF1 and PGF2α found in amniotic fluid and umbilical and placental blood vessels. Initiating factors for parturition (PGF2α and PGE2), inhibit progesterone synthesis and increase estrogen synthesis. PGF2alpha's uterine effect reinforced by oxytocin.
   f. Abortion - induced by PGF2 and PGE2. The 15(S)-15-methyl analogues of PGE2 and PGF2α are more potent.
2. Cardiovascular actions (often complex).
   a. PGE series - fall in blood pressure due to decrease in peripheral resistance (arteriolar smooth muscle relaxation), reflex tachycardia, incease blood flow through vascular beds (many exceptions).
   b. PGF series - PGF2α transitory increase or decrease in blood pressure (species differences), vasoconstriction in many vascular beds (dilation in others), powerful constrictor effect on large veins and pulmonary artery.
   c. PGA series - marked vasodilator and depressor activity.

3. Renal actions (PG synthetase mainly in medulla and PG dehydrogenase mainly in cortex).
   a. PGA2 (medullin), PGE2 and PGF2α in renal medulla released by angiotension II, norepinephrine and sympathetic stimulation. PGE2 directs flow through cortex. PGA2 decreases metabolism and PGA1 stimulates release of aldosterone.
   b. PGs may exert an antihypertensive action -- vasodilators and diuresis with Na+ loss.
   c. PGs may also be concerned with autoregulation in kidney -- maintaining constant blood flow in kidney despite changes in systemic blood pressure.
4. Interactions with adrenergic mechanisms.
   a. PGs released from adrenal medulla and spleen following sympathetic stimulation.
   b. PGE1 and PGE2 inhibit norepinephrine release from sympathetic terminals.
5. Gastrointestinal tract.
   a. Intestinal wall. Contains E and F series ("darmstoff"). Maintenance of tone (?). Longitudinal smooth muscle contraction; circular smooth muscle PGAs and PGFs contraction, PGEs - relaxation. Generally produce intestinal cramps and diarrhea. Vomition with PGF2alpha.
   b. Gastric acid secretion induced by histamine and pentagastrin is decreased by PGEs and PGAs. (15(S)-15- methy PGE2 very powerful). PGE2 present in gastric juice -- modulates secretion.
   c. Intestinal secretion -- increased by PGE1, often with secretory diarrhea.
6. Respiratory tract.
   a. Lungs remove PG's from circulating blood and inactivate them (PG-15-dehydrogenase).
   b. Bronchial smooth muscle -- relaxed by PGEs and contracted by PGF2alpha (reciprocal balance).
   c. PGs released from lungs of sensitized animals following antigen challenge (also with histamine and kinins administration).
7. Lipolysis.

   PGs antagonize lipolytic action of catecholamines and endocrine hormones (ACTH, TSH and glucagon). PGE2 most potent. PGs antagonize adenylate cyclase in adipose tissue.
8. Body temperature.

   PGE1 produces prolonged elevation in temperature; responsible for fever through action on temperature controlling centers in hypothalamus.
9. Inflammatory reactions.

   PGs released with other immediators and intermediators in various types of inflammatory and hypersensitivity reactions. Responsible for several manifestations of inflammatory response. Reactions include vasodilation, increased capillary permeability, edema, leukocyte migration (increased chemotaxis), smooth muscle contraction, local pain. However, responses complex, e.g., PGE1 and PGE2 inhibit histamine release from basophils.

10. Pain.

Mediators of pain-inducing stimuli. Do not directly stimulate sensory receptors but subserve pain by lowering nociceptive threshold for other substances (e.g. serotonin, histamine and kinins).

Thromboxane TXA2 and Prostacyclin (PGI2)

1. Thromboxane A2 synthesized in platelets -- potent inducer of platelet aggregation and constrictor of vascular smooth muscle, thus thrombogenic.
2. Prostacyclin synthesized in intima of blood vessels -- potent inhibitor of platelet aggregation and powerful vasodilator, thus antithrombotic.

Leukotrienes (LTs)

1. Prolonged and pronounced bronchoconstriction (LTC4, LTD4).
2. Inflammatory edema due to vascular permeability (especially venules).
3. Transient contraction of terminal arterioles but potent constrictors of some arteries.
4. Chemotactic factors for neutrophils and eosinophils (LTB4).
5. May release lysozymes from leukocytic granules (LTB4?).

Hydroperoxyeicosatetraenoic acids (HPETEs) and hydroxyeicosatetraenoic acids (HETEs)

1. Chemotactic factors.
2. Enhance release of histamine from basophils.

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Role and Control of Eisosanoid in Acute Non-Immune Inflammation in the Equine

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Abstract

A range of in vitro and ex vivo techniques have been developed and used to study the acute inflammatory process in the horse, with particular reference to the roles of eicosanoids as putative inflammatory mediators and the actions of anti-inflammatory agents as inhibitors of eicosanoid synthesis. Two ethically acceptable models of acute non-immune inflammation based on the inflammatory actions of the galactan, carrageenin, were used to obtain serial samples of inflammatory exudate. Demonstration of the presence in acute inflammatory exudate of PGE2, PGI2, TXA2 and LTB4 suggest that cyclo-oxygenase and lipoxygenase products of arachidonic acid metabolism may be involved in the vascular and cellular components of inflammation. The chemoattractant properties of PGE2 and LTB4 for equine polymorphonuclear leucocytes (PMN) and mononuclear cells (MN) were investigated using two in vitro assay systems. The actions of a range of non-steroidal and steroidal anti-inflammatory drugs on eicosanoid concentrations and cell numbers in acute inflammatory exudate were investigated and their actions were related to drug concentrations in exudate. The effectiveness of circulating concentrations of anti-inflammatory drugs as cyclo-oxygenase inhibitors was assessed by measuring TXB2 concentrations in serum harvested from blood allowed to clot under standard conditions. It is suggested that the use of these techniques, in combination, may help to further our knowledge of the inflammatory process and the actions of anti-inflammatory drugs in the horse.

Introduction

1.1. The inflammatory process

Inflammation can be defined as the response of the living microcirculation and its contents to injury. There are three crucial words in this definition: living, microcirculation and contents. Living tissue responds actively to injury in an attempt to defend the body from the persistencce of the injurious stimulus and the initiation of pathology. The body deploys several defence mechanisms including the immune system. If the initial injury is caused by microorganisms, tissue damage may extend to host cell death. It should be noted that necrotic tissue cannot trigger the inflammatory response. Similarly, avascular tissue cannot respond to injury by producing inflammation. Hence, the importance of the microcirculation, which assists the response to injury in several ways. Vascular endothelial cells change shape and inter-endothelial cell gaps are formed, through which pass plasma proteins. This results in the formation of fluid (protein-containing exudate) in the interstitial space i.e. oedema. The

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contents of the microcirculation comprise not only plasma and its proteins but the cellular elements. In inflammation, phagocytic leucocytes (neutrophils and monocytes) are signalled to leave the blood stream and engulf or immobilise the tissue irritant. These several changes are manifested at the whole animal level as Celsus' classical signs of inflammation, rubor et tumor cum calore et dolore, to which Virchow added the fifth, functio laesa.

1.2. Criteria for identifying mediators of acute inflammation

The ubiquitous nature of eicosanoids and the ease with which they are formed de novo have stimulated many studies of their pharmacological actions and possible roles in physiological and pathophysiological processes since their discovery in 1935 (1). It is clear, however, that much still remains to be learned of their precise roles in, for example, the inflammatory process and the possibility that there may be species difference in eicosanoid synthesis and/or action must also be considered.

In order to establish firmly the role of any substance as a mediator of inflammation in a given species, it is necessary to establish in that species the following criteria (2):

1. Its presence at the site of inflammation in "effective" concentrations and its absence when the reaction subsides.
2. Production of one or more "cardinal signs" when it is introduced into normal tissues.
3. Receptor site antagonists of the substance are anti-inflammatory.
4. depletion of stores or inhibition of synthesis of the substance impairs the inflammatory response.
5. Antagonists of enzymes inactivating the putative mediator enhance the inflammatory response.

Even when all of these criteria have been satisfied, uncertainty may remain concerning the precise role of the newly defined mediator. There are several reasons for this. First, many inflammatory mediators have been identified and there is evidence for a temporal sequence of release and activity, with histamine (and in some species serotonin) mediating early vascular changes, followed by bradykinin and, later still, prostanoids such as PGE2 (3). However, this concept does not take account of the interactions between mediators. The important findings that some prostanoids markedly synergise with bradykinin to enhance its algesic action (4) and with histamine and bradykinin to increase their actions on the permeability of small venules (5) suggest that the principal role of arachidonic acid metabolites may be as modulators rather than mediators. The precise roles of: known mediators like histamine, bradykinin and components of the complement system are also made less certain by the discovery of new putative mediators, such as lipoxins, interleukin I and platelet activating factor (Table 1). Nevertheless, even when more is known about the actions of these compounds, it seems certain that prostanoids such as PGE2 and PGI2 and leukotrienes like LTB4 will remain as major mediators and/or modulators of acute inflammation (Table 1).

In assessing the roles of mediators, differences between acute and chronic forms of inflammation should also be considered. Apart from

lysosomal enzymes, however, virtually nothing is known about the agents which act as mediators of chronic inflammation or of the substances causing the transition from acute to chronic inflammation. This paper will therfore consider only the roles of eicosanoids in the acute inflammatory process and the actions of anti-inflammatory drugs.

1.3. Generation, actions and inhibition of synthesis of eicosanoids

Some of the more important eicosanoids by lipid peroxidation of arachidonic acid are illustrated in Figs. 1 and 2. Arachidonic acid is a 20-carbon unsaturated fatty acid derived from cell membrane phospholipid. All cell types examined thus far, with the exception of erythrocytes, have the capacity to generate eicosanoids. The initial reaction, following an inflammatory stimulus, involves release of arachidonic acid from its esterfield form by the action of phospholipase A2. Released arachidonic acid may serve as a substrate for two enzyme systems. Cyclo-oxygenase generates the unstable cyclic endoperoxide intermediates PGG2 and PGH2 and, by the actions of further specific enzymes, the prostanoids PGE2 and prostacyclin (PGI2) and thromboxane A2 are formed (Fig. 1). The action of lipoxygenase, on the other hand, leads to the synthesis of a series of hydroperoxy- and thence hydroxy-acids and the leukotriene group of eicosanoids (Fig. 2).

Studies of the direct and indirect actions of eicosanoids (Table 1) and their detection in effective concentrations in inflammatory exudate in laboratory animal models of inflammation have indicated that they are amongst the most important mediators and modulators of the acute inflammatory process (6), although a role for these compounds in chronic inflammation has not been firmly established. Their importance to the equine clinician is further indicated by the proposed mode of action of the two most widely used groups of anti-inflammatory agents available for use in the horse (Table 2). The carboxylic and enolic acid groups of non-steroidal anti-inflammatory drugs (NSAIDs) are believed to exert most of their therapeutic actions and side-effects by inhibition of cyclo-oxygenase (7) (Fig. 3). Anti-inflammatory corticosteroids, on the other hand, may act wholly or in part by inducing synthesis of lipocortin, a polypeptide with established anti-phospholipase A2 activity (8). This action of steroids at a more proximal site in the same metabolic pathway provides an attractive theory to explain the wider spectrum of activity of steroids in comparison with NSAIDs. Inhibition of lipoxygenase-derived mediators, such as the potent chemoattractant LTB4, in addition to cyclo-oxygenase inhibition might be expected to block cellular as well as vascular aspects of inflammatory responses.

Interest in the roles of eicosanoids in inflammation has been further stimulated by the search for inhibitors of both of the enzymes which utilise arachidonic acid as a substrate, cyclo-oxygenase and lipoxygenase (Table 2, Fig. 3). These dual inhibitors could provide a novel approach to anti-inflammatory therapy, retaining the wide spectrum of activity of steroids, but hopefully without possessing their side-effects (9).

Other compounds listed in Table 1 (groups 4, 5 and 6) are used in man for the treatment of rheumatoid arthritis, but problems of

toxicity, slow onset of action and the requirement for prolonged therapy (several months of therapy may be required before beneficial effects are obtained), together with the lower incidence of rheumatoid arthritis in animals, have limited the use of these compounds in veterinary medicine. Agents in group 7 produce few adverse effects but their mode of action is unclear, except for orgotein which is known to possess superoxide dismutase activity. Of the other compounds in this group, hyaluronic acid and glycosaminoglycan polysulphate are used to treat chronic degenerative joint diseases but it is doubtful whether they should strictly be classified as anti-inflammatory agents. The present paper is therefore concerned primarily with drugs in groups 1 to 3, since those in classes 1 and 3 are the anti-inflammatory agents used most commonly in veterinary medicine, while the compounds in group 2 could form the next generation of anti-inflammatory agents.

2. Methods for studying inflammation and assessing anti-inflammatory drug activity

Over the last two decades scientists have turned increasingly to models of disease processes to facilitate their studies of disease mechanisms. They have also been used to evaluate the efficacy of both new and existing therapeutic agents. the advantage of such models is that they allow investigations to be undertaken under controlled circumstances and they permit measurements of disease parameters to be made which may be difficult or impossible in clinical patients. A disadvantage, however, is that they will rarely simulate exactly the clinical disease, if only because it will vary considerably, in nature and degree, between one patient and the next.

Several groups have developed models of equine inflammation, involving for example chip fractures, septic arthritis and induced equine myositis. Thus, Jones and Hamm (10) injected lactic acid into four sites on each side of the vertebrae and the resulting inflammatory reaction, which resembled natural 'tying-up' myositis, was assessed by measuring lameness, reduction in stride length, swelling and response to pain. However, in this model the heat generated by increased blood flow was not monitored and inflammatory exudate was not harvested, so that cellular and biochemical changes in the inflammatory lesion were not recorded. We have developed two models of acute non-immune inflammation in the horse, the first based on insertion of polyester sponge strips soaked in carrageenin solution into small subsutaneous pouches in the neck and the second involving injection of carrageenin solutions into subcutaneously implanted tissue cages (11, 12). The former involves an inflammatory reaction of soft tissue which is purely acute, since the reaction is limited to the 24-hour period following the induction of inflammation. The later model also involves induction of an acute reaction but the tissue stimulated is fibrous granuloma tissue; it therefore may be likened to an acute flare-up of a chronic inflammatory lesion.

a. Sponge model

Four polyester sponge strips measuring 50 x 25 x 5 mm and soaked in a sterile 2% solution of lambda carrageenin, were placed in a subcutaneous pouch dissected in the mid-neck region of conscious ponies under local anaesthesia. Sponges were removed serially at pre-

determined times up to 24 hours (usually 4, 8, 12 and 24 hours). Sponges were gently squeezed to permit exudate collection into 2.5 ml heparinised saline. BW 755C (10 ug per tube) was used to prevent artefactual generation of eicosanoids.

b. Tissue cage model

Practice polypropylene golf balls with an internal volume of 35 ml were inserted subcutaneously at prior operation. Fibrous granuloma tissue grew around and into these cages until, after several months, the tissue all but obliterated the cages. At least 3 weeks was allowed following insertion of the cages before an acute inflammatory reaction was induced by injecting 0.5 ml of a sterile 2% carrageenin solution. Cage fluid (inflammatory exudate) samples were collected at pre-determined times up to 48 hours.

In both models of inflammation, the eicosanoids, LTB4, PGE2, TXB2 (the stable breakdown product of TXA2) and 6-keto-PGF1alpha (the stable breakdown product of prostacyclin, PGI2) were monitored by radioimmunoassay, and in some experiments PGE2 was also measured by bioassay using the superficial rat stomach strip (11,12). Exudate samples have also been used to determine biochemical and cellular aspects of acute equine inflammation, including changes in protein and both lysosomal and non-lysosomal enzyme concentrations (11,12,13). In addition, the heat generated at the site of the localized inflammatory reaction has been determined as skin temperature changes by infra-red thermometry. Drug actions and drug distribution were studied, in the models of acute inflammation, by measuring, in cross-over studies, exudate eicosanoid and drug concentrations and exudate leucocyte numbers. In each part of the cross-over, half of the animals received drug treatment and half received no treatment or a placebo.

2.2 Inhibition of platelet cyclo-oxygenase

The relationship between drug concentration in exudate and inhibition of exudate eicosanoid formation is important and it may be useful in predicting dose rates and dosing intervals of anti-inflammatory drugs. However, drug concentrations in plasma may be equally important in determining the ability of drugs to suppress the vasodilator and oedematous components of inflammation. The effectiveness of circulating concentrations of drugs as inhibitors of cyclo-oxygenase was therefore determined by measuring TXB2 concentrations in serum harvested from blood allowed to clot under standardised conditions (at 37^{o}C). By collecting serial serum samples after the administration of a number of anti-inflammatory agents, the time course and nature (competitive or non-competitive) of inhibition of platelet cyclooxygenase was determined.

2.3 In vitro studies of eicosanoids as chemoattractants

a. Preparation of cell populations

Blood was collected from New Forest ponies by jugular venepuncture into lithium-heparin monovettes. PMN and MN leucocytes were separated by Percoll sedimentation as previously described (14). Briefly, 5 ml whole blood was layered onto a Percoll gradient consisting of a 60% Percoll solution (density 1.07 g/ml) over a 75% Percoll solution (density 1.09 g/ml). After centrifugation (200 g for 15 minutes at

4°C), the separated PMN and MN cells were removed, each was washed twice in x1 Hanks Balanced Salt solution, and counted on a ZM Coulter Counter. The cells were re-suspended in Hanks Solution at a concentration of 10^6/ml for the agarose microdroplet assay and at a concentration of 10^6/ml for the millipore assay.

b. Millipore Assay Method

Leuocoyte locomotion was assessed using a modified Boyden chamber techinque (15). 1 ml disposable syringe barrels were cut at right angles to the stem at the 0.55 ml mark and a millipore filter fixed with M.F. cement to the cut end. The PMN or MN cell suspension (0.1 ml) was placed in this upper chamber and a sealed, inverted 5 ml polypropylene syringe used as the lower chamber. Medium containing chemoattractant was added to the lower chamber. Millipore filters of 3 um and 8 um porosity were used for PMN and MN cells, respectively. The incubation time at 37°C was 1 hour for PMN cells and 2 hours for MN leucocytes. After incubation, the filters were removed, fixed in 10% formal saline and stained with haematoxylin. The degree of cell movement was assessed by measuring the distance migrated by the leading front cells.

c. Agarose Microdroplet Assay Method

A modified chemokinesis assay was used (16). Agarose (Indubiose A 37) solution (0.8 w/v in distilled water) was allowed to cool to 37°C and mixed with an equal volume of pre-warmed filtered x1 Hanks Balanced Salt Solution containing 60 mM N-2-hydroxyethylpiperazine-N-2-ethanesulphonic acid (HEPES) at pH 7.3 An equal volume of this diluted agarose-Hanks solution was added to the leucocyte syspension and thoroughly mixed using a Vortex mixer. Aliquots (2 ul) of the cell suspension containing approximately 10^7 cells were pipetted onto the base of each well of a cooled 96 well microtitre plate. After solidification of the agarose droplets (3 to 5 minutes) a total volume of 100 ul x 1 Hanks solution or x1 Hanks solution containing chemoattractant was added to each well. Migration was assessed by planimetry of projected areas after 4 hours incubation for PMN leucocytes and 22 hours for MN cells. Incubation was carried out at 37°C in a 5% C02 humidified incubator.

3. Results and Discussion

Chacterisation of the inflammatory exudate collected from the polyester sponge and tissue cage models of acute inflammation, in terms of biochemical and cellular composition and eicosanoid concentrations, has been reported previously and will not be described in detail in this paper (11, 12, 13, 17). In summary, skin temperature at the site of the lesion increased by 2 to 5°C and exudate protein concentration rose to levels normally occurring in equine plasma. Both lysosomal and non-lysosomal enzymes are also present in exudate (13). Detectable concentrations of the eicosanoids, PGE2, PGI2, TXA2 and LTB4, in exudate have been reported, and the time-course of concentrations changes established (11,12). Exudate leucocyte numbers increased steadily between 4 and 24 hours (the last sampling time) in the sponge model, whereas maximal values were obtained at 12 hours in the tissue cage model. With both models PMN leucocytes predominate, although mononuclear cell numbers do increase with time. Table 3 summarizes

some of the principal advantages and disadvantages of the models.

Table 4 outlines the results of studies of the actions of NSAIDs in the sponge and tissue cage models. Neither phenylbutazone nor flunixin, administered as single doses at recommended dose levels, affected leucocyte numbers in inflammatory exudate, but both agents inhibited the synthesis of PGE2 and PGI2 for 12-24 hours, in spite of relatively short t1/2B values of 4.7 and 1.6h, respectively. This may be due to rapid achievement of plasma drug concentrations in excess of those required to produce 100% inhibition of prostanoid synthesis, but it could also be attributable, in part, to accumulation of phenylbutazone and flunixin in, and relatively slow clearance from, inflammatory exudate. For both drugs exudate : plasma concentration ratios were initially less than one, but by 12 h the ratio was reversed and concentration in exudate continued to exceed those in plasma thereafter. Both drugs thus tended to accumulate in exudate (Table 5).

The conversion of phenylbutazone to an active metabolite, oxyphenbutazone, and the tendency for it also to accumulate in exudate may additionally have contributed to the relatively prolonged action of phenylbutazone (Table 5).

Blockade of synthesis of TXA2 (estimated as serum TXB2 concentration) in blood allowed to clot under standard conditions, was used to study the nature and time-course of platelet cyclo-oxygenase inhibition by circulating anti-inflammatory drugs. Phenylbutazone, flunixin and miloxicam were reversible antagonists, while aspirin produced prolonged, irreversible antagonism (Table 6). The actions of phenylbutazone and flunixin were complete 36-48 hours after the intravenous injection of recommended doses. Miloxicam produced marked inhibition of serum TXB2 synthesis initially, but the effect was not well maintained, TXB2 concentrations having returned almost to normal by 12 hours.

Measurement of drug concentrations in both plasma and exudate together with assessment of the time-course of inhibition of eicosanoids in exudate and of TXB2 in serum may possibly provide an indication of suitable dose rates and dosing intervals for non-steroidal anti-inflammatory agents. The new cycle-oxygenase inhibitor, miloxicam, for example produced marked but transient blockade of serum TXB2 synthesis and only partial blockade of exudate prostanoid and TXB2 concentrations at a dose rate of 0.6 mg/kg. From these findings it is concluded that a moderate increase in dose would probably be required for clinical equine use.

The dual cyclo-oxygenase and lipoxygenase inhibitor BW540C markedly inhibited serum TXB2 production, but only partially blocked TXB2 and prostanoid synthesis in exudate at a dose rate of 100 mg/kg orally (Table 7). This limited effect was probably due to relatively poor penetration of BW540C and its active metabolite BW755C into inflammatory exudate. AUC values for both compounds were almost three times greater for plasma than for exudate. These findings demonstrate the importance of pharmacokinetic principles in designing new anti-inflammatory drugs; modification of structure to facilitate

passage into exudate can be expected to reduce doses required for efficacy with dual inhibitors as with other classes of anti-inflammatory agent.

Moderate inhibition of the heat generated by the acute inflammatory lesion and partial suppression of leucocyte emigration were obtained with 100 mg/kg BW540C. This finding indicates the potential of dual inhibitors as a novel approach to anti-inflammatory therapy, while demonstrating the need to develop compounds of greater potency which will be effective at lower dose rates.

A clinical dose rate of phenylbutazone administered orally resembled BW540C producing partial inhibition of prostanoid synthesis and significant suppression of the rise in lesional skin temperature, but phenylbutazone did not influence exudate leucocyte numbers (Table 4). These findings suggest that recommended doses of phenylbutazone suppress the vasodilation and hence the heat generated in soft tissue inflammation in the horse and indicate that the drug is not merely analgesic, as some clinicians believe. The parallel inhibition of prostanoid synthesis and one of the cardinal signs of inflammation (heat) also provides indirect evidence for a role of prostanoids in mediating or modulating the vasodilator component of acute inflammation in the horse.

A widely though not universally accepted theory of the mode of action of steroidal anti-inflammatory drugs proposes that they induce the synthesis of the polypeptide, lipocortin, which possesses anti-phospholipase A2 activity. The resulting inhibition of arachidonic acid release from its esterified form (bound to cell membrane phospholipid) would be expected to suppress the generation of both cyclo-oxygenase and lipoxygenase derived mediators of inflammation (Fig. 3). This action may explain why steroids can suppress both vascular and cell-mediated components of the inflammatory process. However, in the carrageenin-sponge model of inflammation a clinical dose rate of betamethasone, administered as a single dose or daily for four successive days, failed to inhibit the synthesis of eicosanoids (Table 7). Even with a very high dose rate, inhibition of eicosanoid synthesis did not occur at all time points. Low doses of betamethasone surprisingly and paradoxically increased leucocyte numbers in inflammatory exudate. The cause of this finding is unknown, but it probably cannot be ascribed to a stabilising action of the drug cell or lysosomal membranes, since exudate concentrations of acid phosphatase were also increased. Dexamethasone also failed to inhibit eicosanoid synthesis in the tissue cage model of inflammation. These data fail to support the hypothesis that the anti-inflammatory action of steroids is attributable to inhibition of phospholipase A2.

In parallel with our whole animal studies, we have investigated the chemoattractant properties of two of the eicosanoids detected in the models of equine inflammation, PGE2 and LTB4.

The standard chemoattractants for PMN and MN leucocytes used in this investigation, zymosan activated plasma and N-formyl-methionyl-leucyl-phenylalanine, respectively, produced marked increases in cell

movement in the Boyden chamber millipore assay, relative to those occurring in medium alone. Smaller but statistically significant and concentration related increases in PMN movement were also produced by PGE2 (5 to 500 ng/ml). This eicosanoid also increased MN cell migration, but the response was significant only at the higher concentrations (50 and 500 ng/ml) used in this study.

The chemoattractant actions of PGE2 for equine leucocytes were confirmed by the results of the agarose microdroplet assay. Concentration related increases in droplet area were obtained with PMN cells, whereas MN leucocytes responded only with a concentration of 500 ng/ml.

Increasing effects of LTB4 on PMN movement in the Boyden chamber assay occurred with rising concentrations of 0.1, 1 and 10 ng/ml. A similar graded response was also obtained with this eicosanoid for mononuclear leucocytes, although the lowest concentration used (0.1 ng/ml) produced a slight effect only. Similar results were obtained in the agarose microdroplet assay, and, as with PGE2, PMN cells were more sensitive to LTB4 than MN leucocytes. From this data it seems possible that PGE2 and LTB4 may function as mediators of PMN migration in acute inflammatory processes in the horse, but a similar role for equine MN cells is less likely. Further studies are required to validate these proposals.

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TABLE 1. Inflammatory mediators and their principal properties

KNOWN OR PUTATIVE MEDIATOR	PRINCIPAL PROPERTIES
Histamine	Present is mast cells, basophils, platelets. Released in immune and non-immune inflammation causing vssodilation and increased permeability of small venules.
Serotonin (5-HT)	Like histamine, a simple amine stored in mast cells and platelets. Increases small venule permeability in rodents but may be unimportant in other specimens.
Bradykinin	A nonapeptide synthesised de novo from plasma kinins and inducing vasodilation, increased permeability and pain.
Complement system	Several components (e.g. C3a, C5a, C8, C9) possess pro-inflammatory properties, including vasodilation, increased vascular permeability, leucocyte chemotaxis, mast cell degranulation with histamine release and cytolysis.
EICOSANOIDS a) PGE2	Prolonged vasodilation, potentiation of increased vascular permeability and pain induced by histamine and bradykinin. Usually the predominant prostanoid in acute inflammatory exudate. Limited evidence of chemotactic activity.
b) PGI2	Similar properties to PGE2 with shorter duration of action. Also inhibits platelet aggregation. Chemically unstable forming 6-keto-PGF1alpha
c) TXA2	The major cyclo-oxygenase product in platelets, causing platelet aggregation and vasoconstriction Chemically unstable forming TXB2.
d) LTB4	The most potent chemokinetic and chemotactic factor known for polymorphonuclear leucocytes. Enhances vascular permeability produced by PGE2, PGI2 and bradykinin.
Lipoxin A	Stimulates polymorphonuclear leucocytes to generate superoxide radical and release lysosomal enzymes.
Patelet activating Factor (PAF)	Ether-linked analogue of phosphatidylcholine causing platelet and neutrophil aggregation, bronchoconstriction, vasodilation, increased permeability and chemotaxis.
Interleukin I (IL-1)	Produced by macrophages, possesses chemotactic activity and mitogenic and pyretic properties. Stimulates production of collagenase and PGE2 by rheumatoid synovial cells. May be involved in rheumatoid arthritis.

TABLE 2 Classification of drugs used in the therapy of acute and chronic inflammatory diseases

	CLASS	ADVANTAGES	DISADVANTAGES
1.	Cyclo-oxygenase inhibitors e.g. aspirin, phenylbutazone, miloxicam, flunixin, naproxen	Rapid symptomatic relief from pain, possible suppression of vascular components of inflammation	Little or no effect on cell migration. Gastrointestinal ulceration, blood dyscrasias and other side-effects on kidney and liver
2.	Dual cyclo-oxygenase lipoxygenase inhibitors e.g. BW755C, BW 540C benoxaprofen, timegadine	Experimentally inhibit all aspects of acute inflammation, including cell migration	Some side-effects e.g. hepatotoxicity of some drugs and blood dyscrasias with others
3.	Corticosteroids e.g. dexamethascne, betamethasone, prednisolone	Inhibit but do not abolish all aspects of acute and chronic inflammation	Major side-effects e.g. adrenal atrophy, immune suppression
4.	Slow acting disease modifying agents e.g. penicillamine, gold salts	Some degree of specificity in suppressing rheumatoid arthritis	Very slow onset of actien, possibly severe side-effects
5.	Immune stimulants e.g. levamisole	Selective action in immune-medicated reacticns	Slow acting, doubtful efficacy
6.	Cytotoxic agents e.g. cyclosphosphamide,	Effects can be dramatic in rheumatoid arthritis	Immune suppression, potentially very toxic to rapidly dividing cell populations
7.	Miscellaneous e.g. hyaluronic acid orgotein, GAGPS	Free of major side-effects	Mode of action obscure (except orgotein), administration by intra-articular injection maybe necessary, evidence of efficacy largely anecdotal

TABLE 3 Tissue cage and polyester sponge models of carrageenin-induced acute inflammation: advantages amd disadvantages

ADVANTAGES	DISADVANTAGES
1. Permit the acute inflammatory process and its time course to be studied under controlled conditions.	1. Because of diversity of acute inflammatory conditions encountered clinically, the models cannot simulate closely all clinical conditions
2. Inflammatory reaction of limited duration and confined to a small localised area, causing minimal distress.	2. As models of acute soft tissue inflammation, they do not mimic chronic inflammatory conditions or hard tissue erosion.
3. Exudate collection very simple, permitting analysis, on serial samples, of cellular and biochemical composition and determination of eicosanoid concentrations in exudate.	3. Only provide information on acute inflammatory response to one concentration of a single irritant.
4. Animals recover fully, allowing repetition of experimenral procedures and hence use of cross over design in drug experiments.	4. Provide quantitative information on only one of cardinal signs of inflammation (heat). Redness is not applicable; loss of function is not applicable because of small size of lesion and pain and swelling are assessed only subjectively.
5. Allows studies of drug action on cellular and biochemical composition of exudate, concentrations of eicosanoids and skin temperature to be undertaken.	5. Drug concentration in exudate will not adequately reflect ability to penetrate into all tissues and fluids e.g. synovial fluid.
Permit 'applied' pharmacokinetic studies to be carried out by measurement of drug concentrations in exudate, exudate:plasma concentration ratio and exudate:plasma A.U.C. ratio.	

TABLE 4 Summary of actions of non-steroidal anti-inflammatory drugs in models of carrageenin-induced acute inflammation

MODEL	DRUG	DOSE ADMINISTERED (mg/kg)	COMMON CLINICAL DOSE (mg/kg)	OVERALL EFFECTS ON EXUDATE	
				PROSTANOIDS + TXB_2	LEUCOCYTES
Sponge	Phenylbutazone	4.4 (i.v.)	4.4	Marked inhibition for 12-24 hours	Small, non-significant reductions up to 24 hours
Sponge	Phenylbutazone	3.3 (oral)	2.2 to 4.4	Moderate inhibition for 12 hours	No effect
Tissue cage	Flunixin	1.1 (i.v.)	1.1	Marked inhibition for 12-24 hours	Non-significant reduction at 12 hours
Sponge	Milocicam	0.6 (i.v.)	not established	Moderate inhibition for 12 hours	Small, non-significant reductions up to 24 hours

TABLE 5 Anti-inflammatory drug penetration into inflammatory exudate

A. Exudate: plasma concentration ratio greater than 1 Enolic acids
Phenylbutazone
Oxyphenbutazone (metabolite of phenylbutazone) Miloxicam

Carboxylic acids
Flunixin

Steroids
Betamethasone

B. Exudate: plasma concentration ratio less than 1 Bases
BW 540C
BW 755C (metabolite of BW 540C)

TABLE 6 Percentage inhibition of serum thromboxane synthesis by anti-inflammatory agents

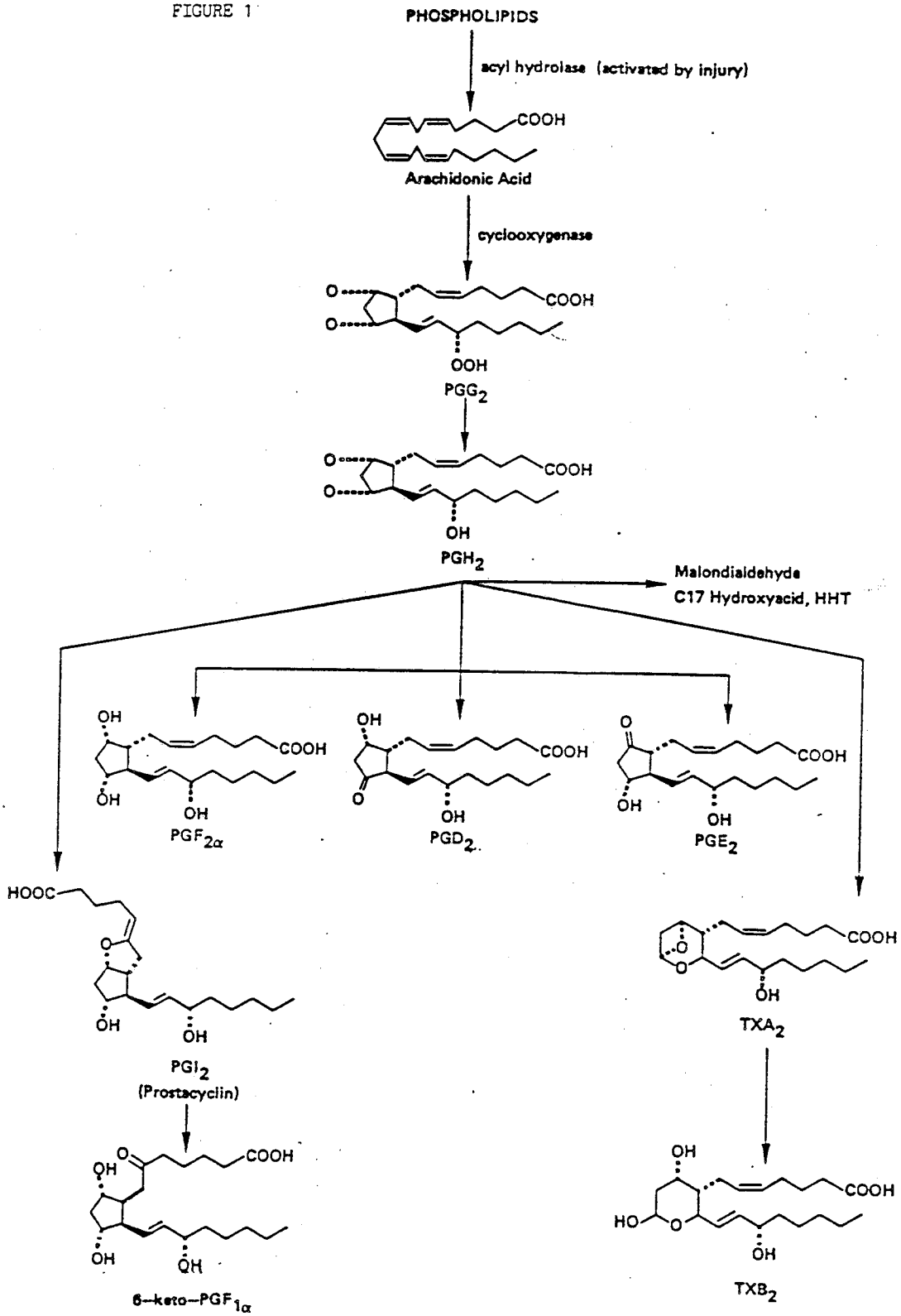
DRUG	DOSE (mg/kg)	ROUTE	PERCENTAGE INHIBITION OF SERUM TXB_2						
			4	8	12	24	2	7	14
			(hours)				(days)		
Aspirin	19.0	i.v.	97	100	99	99	100	100	55
Phenylbutazone	4.4	i.v.	88	75	76	50	7	-25	6
Flunixin	1.1	i.v.	98	85	77	63	-4	-22	-10
Miloxicam	0.6	i.v.	92	72	18	5	-	-	-
BW 540C	100.0	oral	95*	98**	92	76	-	-	-
Betamethasone	0.08	i.v.	9	30	-40	-54	-	-	-

- = not determined
* sample time 3 hours
** sample time 6 hours

TABLE 7 Summary of actions of steroids and dual inhibitors in models of carrageenin-induced acute inflammation

MODEL	DRUG	DOSE ADMINISTERED (mg/kg)	COMMON CLINICAL DOSE (mg/kg)	OVERALL EFFECTS ON EXUDATE	
				PROSTANOIDS + TXB_2	LEUCOCYTES
Sponge	Betamethasone	0.08 (i.v.)	) 0.08	No inhibition	Increased at 8 and 12 hours
Sponge	Betamethasone	0.08 daily for four days (i.v.)	)	Small but inconsistent degree of inhibition	Increased at 12 hours
Sponge	Betamethasone	1.0 (i.v.)	)	Inhibition at some time points	Decreased but only at 24 hours
Tissue cage	Dexamethasone	0.06 (i.v.)	0.02	No inhibition	No effect
Tissue cage	BW 540C	100.0 (oral)	not established	Moderate inhibition of TXB_2 but not PGI_2	Decreased but only at 24 hours

FIGURE 1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COOH
OH
PGH2
Malondialdehyde
C17 Hydroxyacid, HHT
OH
OH
COOH
OH
PGF2α
OH
O
COOH
OH
PGD2
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(Prostacyclin)
O
O
COOH
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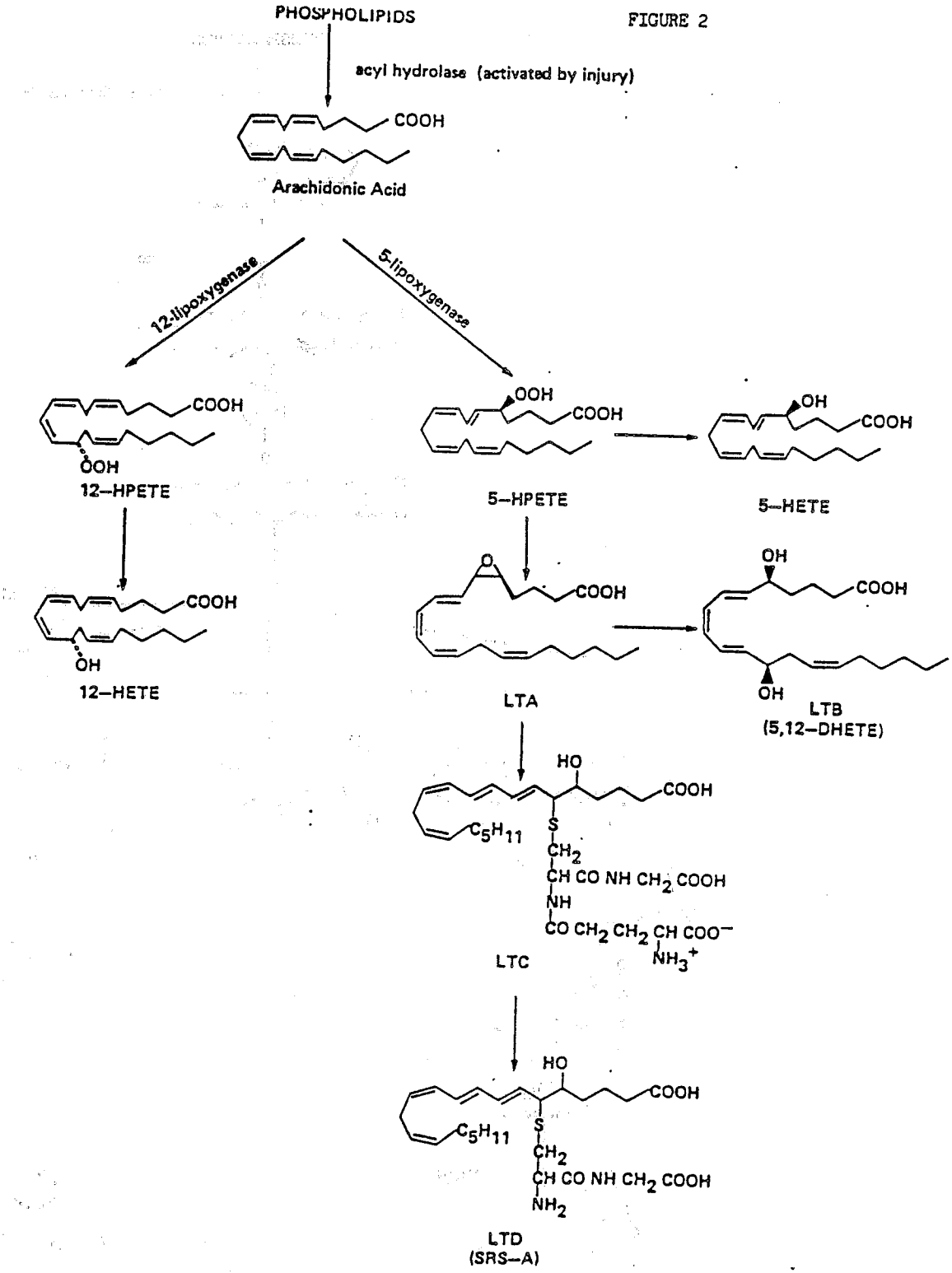
TXB2

FIGURE 2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COOH
C_5H_{11}
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CH_2
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NH
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NH_3^+
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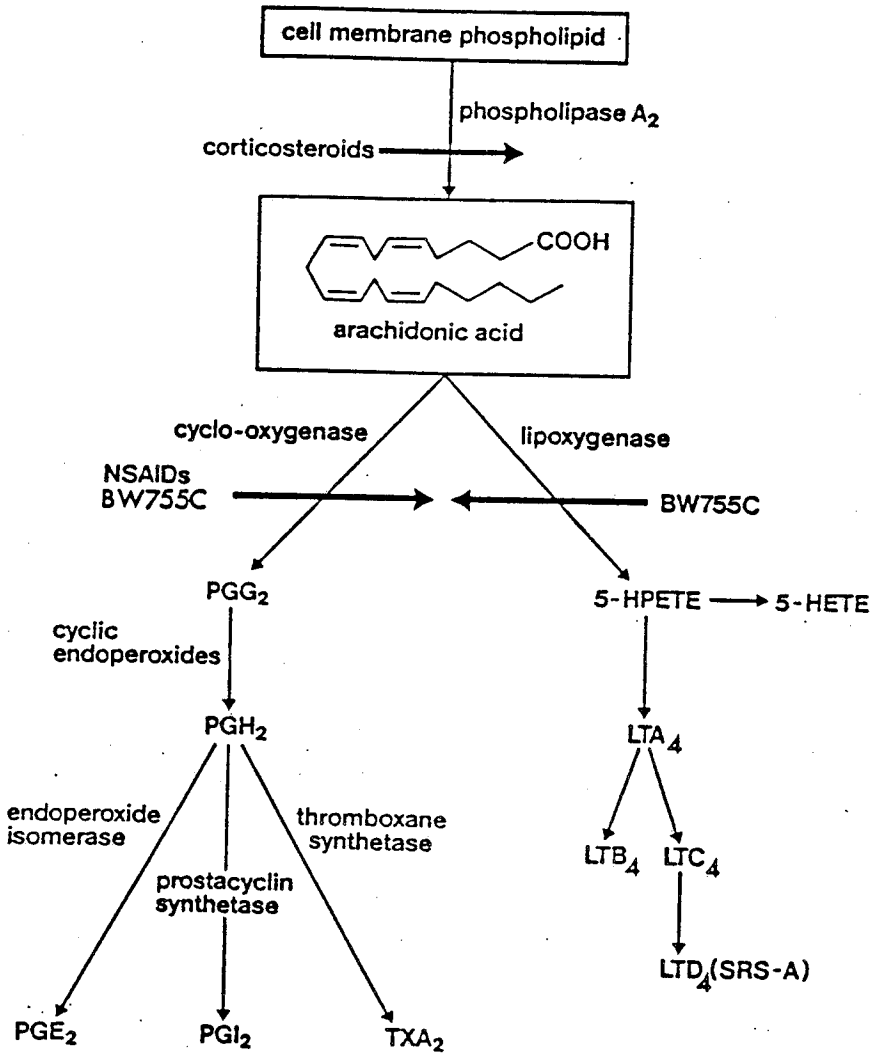
FIGURE 3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endoperoxides
PGH_2
LTA_4
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thromboxane
synthetase
prostacyclin
synthetase
LTB_4
LTC_4
LTD_4(SRS-A)
PGE_2
PGI_2
TXA_2

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Eicosanoids and Ocular Inflammation

Lloyd N. Fleisher

Introduction

Uveitis is a descriptive term for inflammation of the iris, ciliary body, and choroid, i.e., those tissues which make up the uveal tract. Anterior uveitis encompasses inflammation restricted to the anterior uvea (the iris and ciliary body), whereas posterior uveitis includes the posterior uvea (the choroid). Anterior uveitis is the more common of the two and this discussion will primarily address this form of ocular inflammation.

Uveitis can be induced by a variety of factors, many of which are poorly understood. Inflammation may be secondary to infections of the cornea, general systemic infections, injury to the eye, cataract surgery, trabeculoplasty, etc. In many cases, uveitis is part of a more generalized syndrome, such as rheumatoid arthritis, Behcet's disease, ankylosing spondylitis, Reiter's syndrome, sarcoidosis, etc. However, in many cases the exact cause of the uveitis is uncertain. Regardless of the etiology, anterior uveitis is characterized by pain, engorgement of the iridal blood vessels (hyperemia), alterations in intraocular pressure (IOP), disruption of the blood-aqueous barrier and the subsequent appearance of elevated levels of plasma proteins in the aqueous humor (aqueous flare), miosis, infiltration of leukocytes into the anterior chamber, and the appearance of inflammatory mediators in the intraocular fluids (IOFs). The infiltration of leukocytes into the anterior chamber has been used as a criterion to differentiate anterior uveitis from the ocular response to various physical insults, such as paracentesis. Although paracentesis does not usually result in leukocyte infiltration, most of the other signs of inflammation are present and many investigators view the response to paracentesis as a mild form of inflammation.

Considerable interest has focused on the role of biochemical mediators of the ocular inflammatory response, since the pharmacologic manipulation of these substances, in particular the inhibition of synthesis and/or blockade of action, could attenuate the inflammatory process and the damage to ocular tissues that often accompanies periods of prolonged uveitis. Damage to ocular tissues can be particularly hazardous, since the clarity of the cornea, lens, and IOFs and the integrity of the retina are essential for normal vision.

The involvement of prostaglandins (PG) as ocular inflammatory mediators was first suggested by Ambache et al. who observed that mechanical irritation of the iris, collapse of the anterior chamber, or movement of the lens caused the release of Irin (a mixture of E and F PGs; 1) into the rabbit aqueous humor. In 1972, Eakins et al. detected the presence of PGE-like activity in the aqueous humor of rabbits with immunogenic uveitis by the intravitreal injection of bovine serum albumin (2). Similar results were obtained following

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ocular inflammation induced by the intravitreal injection of bacterial endotoxin (3-5). Eakins has described four sets of observations which support the concept of PGs as mediators of the ocular inflammatory response (6). These observations can now be extended to include the lipoxygenase products of arachidonic acid metabolism, i.e., the leukotrines (LT) and various hydroxylated forms of arachidonic acid, the HETEs:

1. Ocular tissues can synthesize ecosanoids (i.e., PGs, LTs, and HETEs).
2. Elevated levels of eicosanoids have been detected in the aqueous humor and increased production of eicosanoids by ocular tissues has been observed during ocular inflammation in experimental animals and man.
3. Eicosanoids can reproduce some of the signs of ocular inflammation when applied topically, intracamerally, or systemically.
4. Eicosandoid synthesis inhibitors can reproduce some of the signs of ocular inflammation.

Synthesis of PGs Ocular Tissues

It has been clearly demonstrated that the iris-ciliary body synthesizes a variety of cyclooxygenase products, including PGE2,PGF2,PGD2, 6-keto-PGF1alpha (inactive metabolite of PGI2), and TxB2 (inactive metabolite of TxA2; 7-9). Significant biosynthesis of PGs has also been demonstrated in other ocular tissues, including the cornea, conjuctiva and recently, the lens, and cultured cells of the trabecular meshwork (5,7,8,10-12). In terms of relative cyclooxygenase activity, the iris-ciliary body and conjuctiva are most active, the cornea and trabecular cells are intermediate, and the lens least active.

Recent reports indicate that certain ocular tissues can synthesize lipoxygenase products of arachidonic acid metabolism. Synthesis of 5-HETE, 12-HETE, and LTB4 from exogenous 14-C-arachidonic acid has been demonstrated for the anterior uvea and conjuctiva from a number of species (13,14). Synthesis of the slow substances of anaphylaxis (SRS-A; a mixture of LTC4, D4, and E4) has been detected for rabbit anterior uvea and conjuctiva (15). Homogenates of rabbit and frog cornea can convert 14-C-arachidonic acid into 12-HETE (16). Substantial quantities of both 5 and 12 lipoxygenase products were synthesized from 14-C-arachidonic acid by cryogenically lesioned rabbit corneas (17). The authors did note that some of the lipoxygenase metabolities could arise from inflammatory cells attracted to the wound area.

The capacity of the lens to synthesize lipoxygenase product is still an open question. Williams et al. was unable to detect lipoxygenase activity in rabbit or frog lens (16). However, reports of LTB4 production by cultured bovine lens, 5-HETE production by lenses from estrogen-treated rabbits, and 5- and 15-HETE production by mouse lens epithelial cells have recently appeared (18-20).

Elevated Eicosanoid levels in the IOFs and Increased Eicosanoid Production by Ocular Tissues are Associated with Inflammation.

Elevated of PGs have been detected in inflammatory exudates from

a variety of tissues and it has become quite clear that PG levels are significantly elevated in the aqueous and vitreous humor of inflamed eyes (21-23). PGs (mostly PGE2) are released into the aqueous humor during immunogenic ocular inflammation induced by the intravitreal injection of bovine serum albumin and during ocular inflammation induced by the intravitreal or systemic administration of bacterial endotoxin (2-5). PGs have also been detected in the aqueous humor of humans with acute anterior uveitis and Behcet's disease (24,25). It should be noted that unlike other tissues, the anterior uvea does not enzymatically degrade PGs to any significant extent (26). This has been attributed to a deficiency in 15-PG-dehydrogenase and 13, 14-reductase activities. A PG transport system has been demonstrated in the anterior uvea and the ability of this system to transport PGs is impaired during ocular inflammation (27). It is not clear whether the accumulation of PGs in the aqueous humor during inflammation is due to an impairment of this transport system, to increase production of PGs by the inflamed ocular tissues, or to a combination of these factors. It should be kept in mind that the inflammatory cells which invade the anterior chamber during the ocular inflammatory response are also a rich source of eicosanoids.

Marked increases in the production of PGs by ocular tissues occurs during the ocular inflammatory response. PG synthesis by the iris-ciliary body was significantly increased during ocular inflammation induced by the intravitreal injection of Shigella or E. coli endotoxin (5,28,29). Recently, it has been demonstrated that increased lenticular synthesis of PGE2 and PGF2 is associated with ocular inflammation induced by the intravitreal injection of E. coli endotoxin (5,30). In this study, the levels of PGE2 in the aqueous humor correlated with lenticular PGE2 synthesis and the number of leukocytes in the aqueous humor during the time interval when ocular inflammation was most intense (from 18 hours to 2 days post-endotoxin injection). This suggests that both the lens and the leukocytes may contribute to the elevated PGE2 levels in the aqueous humor in this experimental model of ocular inflammation.

Far less information exists on the levels of lipoxygenase metabolites in the IOFs ocular inflammation. Elevated levels of PTB4 and C4 have been detected in the aqueous humor from inflamed rabbit and guinea pig eyes (31-33). Preliminary results in this laboratory indicate that 5-HETE and LTC4 are significantly elevated in the aqueous humor from rabbit eyes 24 hours after the intravitreal injection of E. coli endotoxin (34). Whether these lipoxygenase metabolites arise from ocular tissues or from the inflammatory cells that invade the anterior chamber is unclear at that time.

In a recent study in humans, significant levels of LTs B4, C4, D4, and E4 were detected in the aqueous humor from inflamed and normal eyes. However, no marked differences in LT levels were observed between the normal and inflamed eyes (35).

Eicosanoids Can Induce Some of the Signs of Ocular Inflammation.

The observations that PGs were associated with ocular inflammation came at a fortuitous time, since pure preparations of PGs

also became available during the same time span. A prodigious effort then ensued, with the implicit assumption that application of PGs to the eye could induce an acute inflammatory response. Over 20 years have elapsed since these investigations were initiated and there is still no consensus on precisely what role eicosanoids play in the ocular inflammatory response. Some of the factors contributing to this confusion are:

1. Species differences in responsivity.
2. The route of administration.
3. The dose administered.
4. The means by which inflammation is induced.
5. Criteria used to evaluate the response (e.g., changes in pupil size, IOP, etc.).
6. The assumption that the application of eicosanoids to a normal eye exerts the same effect as do eicosanoids released in situ during inflammation.

Despite this confusion, eicosanoids do have well documented effects when applied to the eye. Administration of PGs, either topically, intracamerally or intravitreally, affects ocular blood flow and the blood-aqueous barrier, pupil size, and IOP (6,36-40). Furthermore, intracameral injection of LTB4 stimulates the migration of leukocytes into the aqueous humor, while LTC4 and D4 constrict the pupil (41-43). In a separate study, topically applied LTD4 produced an increase on conjuctival micropermeability, but did not affect the blood-aqueous barrier (44).

It should be appreciated that the ocular inflammatory response is mediated by a number of substances, many of which seem to exercise redundant actions on the physiology of the eye. Thus, it is not surprising that some inflammatory events, in particular pupillary constriction, are due to the release of substance P from the trigeminal nerve (45-48). Although PGs can induce miosis, especially when administered intracamerally, the neuronal release of SP mediates in large part, if not entirely, the constriction of the pupil in inflamed eyes (6,49).

The role played by PGs in the normal maintenance of IOP and in the alterations in IOP that occur during inflammation has received considerable attention. Most of the early work indicated that intracameral or topical administration of PGs or arachidonic acid usually increased IOP (6,36,50-52). However, in more recent studies it has been difficult to demonstrate a clear hypertensive action for PGs. In fact, in most cases, topical administration of low doses of PGE2 or PGF2alpha has produced a reduction in IOP (39,53-55). This has promoted intense interest in the use of PGs as anti-glaucoma agents. However, side-effects such as conjuctival hypermia, ocular smarting, and headaches have limited the usefulness of these agents. The development of new PGF2 analogs may help to circumvent these problems.

The precise effects that endogenously released PGs exert on IOP during inflammation is unclear. During inflammation induced by the

corneal administration of nitrogen mustard, there is a biphasic increase in IOP. The initial hypertony, which is unaffected by cyclooxygenase inhibition, but blocked by denervation of the eye, occurs within minutes of the irritative insult and peaks within 40 minutes. A prolonged period of normo- or hypotension then ensues, which is followed by a secondary hypertension (after 3-12 hours), which is blocked by cyclooxygenase inhibition, but is affected by ocular denervation (47). It appears that the ocular hypertension observed in this model of inflammation is mediated both by PGs and a neurogenic mechanism.

Perhaps the single aspect of ocular inflammation most clearly modulated by PGs is iridal hyperemia and to a lesser degree, distruption of the blood-aqueous barrier. Topically applied PGs, especially vasodilator PGs such as PGE2 and PGI2, increase ocular blood flow, and induce engorgement of the vasculature in the anterior uvea and the appearance of elevated levels of plasma proteins in the aqueous humor (6,38,49,56). It has been suggested that the vasodilatory PGs work in concert with LCT4 and D4 (both of which increase microvascular permeability) to produce the disruption of the blood-aqueous barrier (57,58). However, there is little direct experimental evidence to support this hypothesis.

Perhaps the most poorly understood aspect of ocular inflammation concerns the role of eicosanoids in the cellular invasion of the anterior chamber. There is general agreement that PGs do not exert a major influence on leukocyte chemotaxis. Much of the data supporting this assertion comes from studies utilizing nonsteroidal anti-inflammatory agents, such as aspirin or indomethacin. With the possible exception of PGE1. which is not synthesized by mammalian tissues to any significant degree, PGs have not been shown to stilulate the migration of leukocytes into the anterior chamber (40,49,54,55). On the other hand, LTB4 is known to be a powerful leukocyte chemotactic agent and potently stimulates the migration of leukocytes into the anterior chamber when administered intracamerally (41,42,57). However, until more specific lipoxygenase inhibitors become available, it is impossible to determine whether release of LTB4 provides the stimulus for leukocyte migration into the anterior chamber during the ocular inflammatory response.

Inhibition of Eicosanoid Biosynthesis Can Block Certain Signs of Ocular Inflammation

If eicosanoids control aspects of the ocular inflammatory response, then inhibition of eicansoid biosynthesis should prevent these inflammatory sequelae. The corticosteroids have been the cornerstone of therapy for the treatment of various forms of anterior uveitis, and at least part of their efficacy is probably due to this inhibition of arachidonic acid mobilization (59,60). However, corticosteroid therapy can result in serious complications, particularly in the so-called steroid responder, where serious and potentially sight-threatening increases in IOP may occur in ocular inflammation associated with an infectious agent, especially when that agent is a virus (61-63). Many of the nonsteroidal anti-inflammatory drugs (NSAIDS) exert an anti-inflammatory action, but do not appear to

share the side-effects of corticosteroid therapy (64). In an early study, Neufeld et al. showed that systemic administration of aspirin markedly reduced the disruption of the blood-aqueous barrier in rabbits following a variety of irritative stimuli (65). Following paracentesis in the rabbit, Miller et al. found that aspirin reduced the disruption of the blood-aqueous barrier and this effect correlated with the decrease in aqueous humor PGE2 levels (66). Topical administration of indomethacin in rabbits has afforded similar protection against disruption of the blood-aqueous barrier following ocular inflammation induced by intravitreal injection of bovine serum albumin (67). In a comprehensive study of ocular inflammation in the rat, Bhattacherjee et al. found that flurbiprofen and the dual cyclooxygenase/lipoxygenase inhibitor BW755c, administered orally or topically, were as effective as dexamethasone in inhibiting iridal hyperemia and cellular accumulation in the anterior chamber (68). Indomethacin and benoxaprofen were effective against cell migration, but only at very high doses. However, in another study, systemic administration of flunixin or indomethacin, but not BW755c, reduced the disruption of the blood-aqueous barrier in the intravitreal endotoxin model of ocular inflammation (69). Topically applied indomethacin and tolmetin have proven quite effective as anti-inflammatory agents in experimental immunogenic uveitis (3,70). Furthermore, treatment with indomethacin prevents or partially reduces the rise in IOP that occurs after experimental corneal burns, topically applied nitrogen mustard, or topically applied arachidonic acid (47,71-73).

Since all NSAIDS are cyclooxygenase inhibitors, it is reasonable to assume that at least part of the anti-inflammatory activity exerted by these agents during experimentally-induced ocular inflammation is due to inhibition of PG biosynthesis. The NSAIDS have also been used in the treatment if endogenous anterior uveitis and inflammation secondary to ocular trauma. The anti-inflammatory activities of topical tolmetin, predisolone, and betamethasone were evaluated in patients with acute endogenous nongranulomatous uveitis. No significant differences were observed in the resolution of the inflammation over a 21-day period with any of the drug treatments (74). The authors suggested that the improvement seen in these patients was due to atropine and/or the natural behavior of the disease. However, since there was no placebo group, it is difficult to draw definitive conclusions from this study. However, in two uncontrolled case reports of acute uveitis which could not be controlled by aspirin, indomethacin, or corticosteroid therapy, ibuprofen provided rapid improvement and long-term protection against recurrence of the inflammatory event (75).

NSAIDS have been employed extensively to minimize postsurgical inflammation following argon laser trabeculoplasty and intraocular surgery. However, both Tuulonen and Pappas et al. reported that topical application of indomethacin did not protect against the increase in IOP that often follows argon laser trabeculoplasty (76,77). On the other hand, both Sanders et al., using topically applied indomethacin, and Schrems et al. using systemically administered indomethacin aspirin found that these NSAIDS protected

against the disruption of the blood-aqueous barrier following argon laser trabeculoplasty (78,79). Furthermore, topical indomethacin was protective against disruption of the blood-aqueous barrier and acted synergistically with topical corticosteroids to prevent the breakdown of the blood-aqueous barrier following the removal of cataractous lenses and the insertion of lens implants (78,80,81). These studies underscore the importance of PGs in the control of ocular blood flow and the integrity of the blood-aqueous barrier, and the somewhat ambiguous importance of PGs in the control of IOP.

Comments and Conclusions

It has probably become obvious by this point that although there is an abundance of information on eicosanoids and ocular inflammation, the precise roles played by these autacoids during the ocular inflammatory response are still poorly understood. Perhaps the most widely accepted role for the PGs is in the control of ocular blood flow and the maintenance of the integrity of the blood-aqueous barrier. Precisely what role, if any, the eicosanoids play in the control of pupillary diameter, the IOP, and in the attraction of cells to the anterior chamber, is still largely unresolved. There is little dispute that exogenously applied PGs can affect the IOP; however, there is no hard evidence to show that PGs actually regulate the IOP during the ocular inflammatory response. It should be noted that although eicosanoids have traditionally been thought of as inflammatory mediators, with the implicit assumption that their ocular effects are pro-inflammatory, their overall function may actually be anti-inflammatory. PGs have been proposed to exert a negative feedback on the functions of inflammatory cells (82,83). Furthermore, PGE1 and PGE2 have been shown to inhibit the release of LTB4 and superoxide anion from elicited rat peritoneal and human peripheral neutrophils (84). This effect was associated with increased levels of cyclic AMP and these PGs are known to activate adenylate cyclase in many types of cells (82). In an elegant series of experiments on the effects of intravitreally administered PGs on ocular permeability, Wong and Howes found that if PGE1, PGE2, and to a lesser degree PGF2, were injected into the rabbit eye intravitreally, then the disruption of the blood-aqueous barrier produced by the intravenous administration of bacterial endotoxin 12-18 hours after PG injection was markedly reduced (85). In other words, these PGs appeared to exert an anti-inflammatory action, as evidenced by a blunted increase in ocular permeability during ocular inflammation. PGs may thus exert a modulating effect on the ocular inflammatory response. This should be kept in mind as new and more specific lipoxygenase and dual cyclo-oxygenase inhibitors are developed and introduced as therapeutic agents.

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