REVIEW ARTICLE

The eicosanoids and their biochemical mechanisms of action

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Introduction

Low dosages of aspirin [1,2] and ingestion of certain fish oils [3–6] have recently been touted as having some value in preventing cardiovascular disease. Aspirin is a nonsteroidal anti-inflammatory drug which exerts its action by inhibiting the synthesis of prostaglandins. The abundant ω3 polyunsaturated fatty acids of fish oils derived from cod and salmon also affect the synthesis of prostaglandins, as well as the synthesis of the leukotrienes and related hydroxy fatty acids; the effects of ω3 fatty acids on prostaglandin and leukotriene synthesis result from the ability of ω3 fatty acids to replace the more common ω6 polyunsaturated fatty acids at the *sn*-2 position of glycerophospholipids, which are precursors of the eicosanoids. Actually, the benefits of prophylactic use of aspirin and fish oils by healthy individuals are largely unproven. For most people, the minuses of aspirin ingestion probably outweigh the pluses [7–9]. However, there are real possibilities that changing the normal patterns of eicosanoid production can be useful in ameliorating certain forms of cardiovascular disease [7–15], osteoporosis [16,17], and arthritis [18]. Certainly, simple aspirin ingestion is already established as a useful treatment for unstable angina [14,15].

The prostaglandins, leukotrienes and related hydroxy fatty acids are members of a group of compounds collectively known as the 'eicosanoids'. This review is designed to provide an overview of the eicosanoid area. I will begin by indicating what compounds are considered to be eicosanoids; I will then proceed to a discussion of the pathways involved in the biosynthesis of these compounds; and finally, I will conclude by discussing how eicosanoids act at the molecular level to elicit their effects. I plan to place particular emphasis on discussing the biochemical mechanisms of action of eicosanoids, since this topic has generally been given little attention. I plan to develop the concept that all the various biological actions of eicosanoids can be understood as being initiated by the interaction of an eicosanoid with a receptor which is coupled to a guanine nucleotide regulatory (G) protein.

What are eicosanoids and where are they found?

First, some definitions. The term 'eicosanoid' has evolved to denote a large, and still growing, family of oxygenated C_{20} fatty acids (Fig. 1). The eicosanoid family is made up of three clans, which include the prostanoids (prostaglandins and thromboxanes) which are synthesized via the 'cyclo-oxygenase' pathway, the leukotrienes and certain mono-, di- and tri-hydroxy acids which are formed via 'lipoxygenase' pathways, and the epoxides which are formed by a cytochrome *P*-450 'epoxygenase' pathway.

Eicosanoids are synthesized from naturally occurring

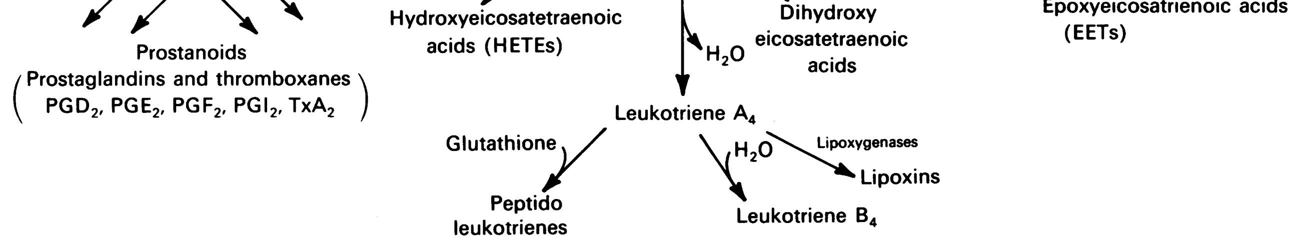

Fig. 1. The arachidonate cascade

Pathways involved in oxygenation of arachidonic acid leading to the production of eicosanoids.

Abbreviations used: PG, prostaglandin; Tx, thromboxane; LT, leukotriene; (di)HETE, (di)hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; AVP, [arginine]vasopressin; GTPγS, guanosine 5'-[γ-thio]triphosphate; fatty acids are designated using the omega (ω) nomenclature [R. T. Holman (1966) Prog. Chem. Fats Other Lipids 9 (part I), 3–12], which designates fatty acids according to chain length, number of double bonds and position of the double bond nearest to the terminal (ω) methyl group (at the opposite end from the α-carbon, which is linked to the terminal carboxyl group). This nomenclature assumes skipped unsaturation and *cis* geometry of double bonds.

C_{20} polyunsaturated fatty acids containing three to five *cis*, methylene-interrupted double bonds. These acids include 8,11,14-eicosatrienoic acid [19,20] and 5,8,11,14-eicosatetraenoic acid (arachidonic acid; [21,22]) which are members of the ω6 family of essential polyunsaturates, and 5,8,11,14,17-eicosapentaenoic acid [3,5,20], the well-known 'fish oil' fatty acid (i.e. 'EPA') which is a member of the ω3 family. Leukotrienes, but not prostaglandins or thromboxanes, can also be synthesized from 5,8,11-eicosatrienoic acid, an ω9 fatty acid which accumulates in essential fatty acid deficiency [23]. The major precursor in most mammalian systems is the most abundant C_{20} polyunsaturate, arachidonic acid (Fig. 1). Prostanoids and lipoxygenase products of the eicosanoid family are formed by both vertebrates and invertebrates, but not by plants or bacteria which lack appropriate polyunsaturated C_{20} fatty acid precursors [24,25].

A limitation of the term 'eicosanoid' is that certain C_{18} and C_{22} fatty acids, including octadecadienoic acid (linoleic acid, 18:2ω6), docosapentaenoic acid (adrenic acid, 22:5ω6) and docosahexaenoic acid (22:6ω3) can be converted to eicosanoid homologues [20,24,26] which are not, strictly speaking, eicosanoids. Oxygenated C_{18} fatty acid products related to the eicosanoids are also formed from linoleic acid by plants via plant lipoxygenases [27,28]. The lipoxygenase pathways in plants and animals are related. Rat 5-lipoxygenase has marked sequence similarities to the soybean 15-lipoxygenase [28,29]. My description of the eicosanoids will be limited to the cyclo-oxygenase and lipoxygenase pathways. The epoxygenase pathway has received considerable attention over the past several years and certain epoxygenase products clearly have potent biological activities, including effects on platelet aggregation and ion and water transport [30–34]; however, there is still a lack of compelling evidence that these compounds are formed *in vivo*.

How are prostanoids, leukotrienes and related hydroxy fatty acids synthesized?

Prostanoid synthesis: an overview. An outline of salient features of the cyclo-oxygenase biosynthetic pathway is as follows. Prostanoids are formed in three stages (Fig. 2; [35]): (*a*) release of arachidonic acid from precursor glycerophospholipids, (*b*) oxygenation of free arachidonic acid by prostaglandin endoperoxide G/H (PGG/H) synthase (cyclo-oxygenase), and (*c*) metabolism of PGH_2 to a specific biologically active endproduct PGE_2, $PGF_{2\alpha}$, PGD_2, PGI_2 (prostacyclin), or thromboxane A_2 (TxA_2).

Arachidonic acid release. Prostanoids are not stored by cells, but rather are synthesized in response to cell-specific proteolytic or hormonal stimuli. For example, platelets form prostanoids (primarily TxA_2) in response to thrombin or collagen [36–39], while other cells respond to these or other agents such as angiotensin II [40], bradykinin [40–42], and [arginine]vasopressin (AVP) [42–44]. An immediate effect of each of these stimuli is to increase the concentration of free arachidonate in the vicinity of PGG/H synthase. Normally, cells make little or no prostanoid, and increases in prostanoid production are temporally correlated with arachidonate 'release', suggesting that this is an important control point.

The biochemical details of the events involved in arachidonate release have not been resolved. It seems clear that the process is usually receptor-mediated and that many types of hormones, autocoids, growth factors, and tumour promoters can elicit arachidonate release [36–46]. However, prostanoid formation can also be elicited by mechanical stresses on cells, such as shear forces acting on vascular endothelial cells [47], and responses to these stresses may not be receptor-mediated. Arachidonate release happens relatively quickly (i.e. in 5–60 s), and is typically accompanied by turnover of inositol-containing phospholipids [36–39,48,49]. In fact, some of the arachidonate used for prostanoid formation may be derived from the sequential hydrolysis of phosphoinositides by phospholipase C and diacylglycerol lipase [36,50]; however, the major sources of released arachidonate are probably the most abundant glycerophospholipids, phosphatidylcholine and phosphatidylethanolamine, and the key enzyme is probably a phospholipase A_2 such as the one from the P388D_1 macrophage line described by Dennis and his coworkers, which is sensitive to specific inhibition by arachidonic acid [50–52].

Prostaglandin endoperoxide synthesis. Conversion of arachidonic acid to the prostaglandin endoperoxide PGH_2 is mediated by PGG/H synthase, an integral membrane protein found in greatest abundance in the endoplasmic reticulum of prostanoid-forming cells [35,53,54]. The detergent-solubilized enzyme appears to be a dimer composed of identical subunits [55,56]. PGG/H synthase exhibits two distinct catalytic activities, a bis-oxygenase (cyclo-oxygenase) involved in PGG_2 formation and a hydroperoxidase mediating a net two-electron reduction of the 15-hydroperoxyl group of PGG_2 to yield PGH_2 [57,58]. Both activities require haem [55,57,59–61]. The cyclo-oxygenase, but not the hydroperoxidase activity, is specifically inhibited by aspirin and related nonsteroidal anti-inflammatory drugs [62–64]. Upon exposure to aspirin, the enzyme is *O*-acetylated at a serine residue located 70 amino acids from the *C*-terminus [65,66]; acetylation leads to irreversible cyclo-oxygenase inhibition [67], and thus, new enzyme synthesis is required before more prostanoids can be produced. Indomethacin, meclofenamate and flurbiprofen also cause irreversible inactivation of the cyclo-oxygenase activity but apparently without covalent modification of the enzyme [68,69]. Other common nonsteroidal anti-inflammatory drugs, such as ibuprofen, flufenamic acid, and sulindac, are reversible enzyme inhibitors which are competitive inhibitors of arachidonate binding [68,70].

The level of PGG/H synthase protein has been shown to be influenced in various cell and organ systems by steroids [71,72], growth factors [45,72–75] and tumour promoters [76], suggesting that regulation of the level of this enzyme is an important part of regulating prostanoid formation. In mouse 3T3 cells, where prostaglandins are required for replication, PGG/H synthase gene expression is induced by serum growth factors [78]. Induction is relatively rapid (about 2 h), occurring at the time of induction of immediate early protooncogenes such as c-*fos* and c-*myc*. Thus, the PGG/H synthase gene appears to be an important cell-cycle-regulated gene. Efforts are underway in several laboratories to isolate and sequence this gene.

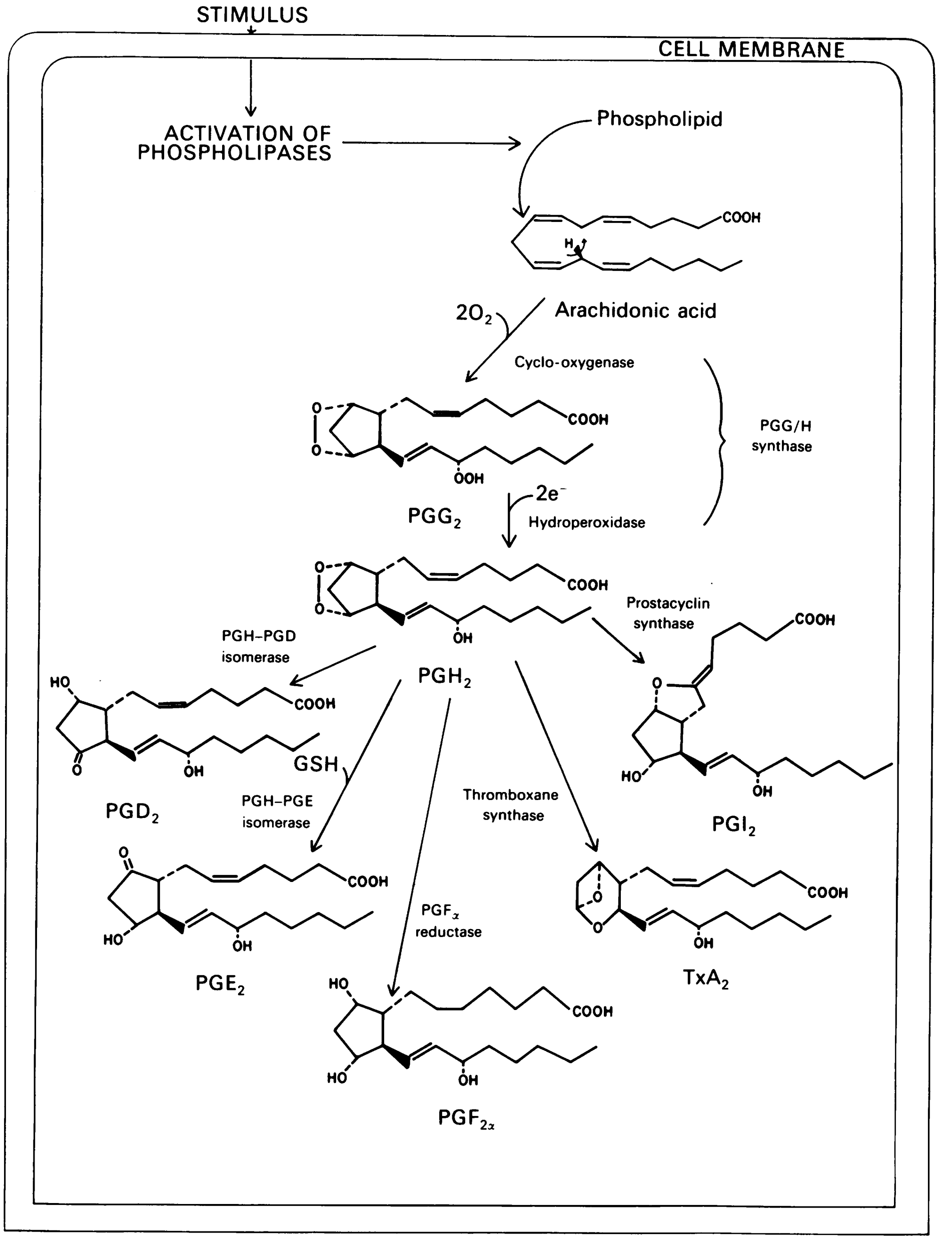

Fig. 2. Biosynthetic pathway for prostanoid formation

Prostaglandin endoperoxide metabolism. The biologically active prostanoids, which are considered to be PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2 and TxA_2 (and possibly PGH_2), are synthesized in a cell-specific manner from PGH_2. That is, any given prostanoid-forming cell tends to form only one of these compounds as its major product [25,35]. For example, smooth muscle cells and endothelial cells from large arteries form primarily PGI_2 [35,78–80], blood platelets form mainly TxA_2 [81], PGE_2 is the major product of collecting tubule cells [43], and $PGF_{2\alpha}$ is the product of uterine endometrium [71].

In comparison to PGG/H synthase, relatively little is known about the enzymes which catalyse the metabolism of PGH_2. Both PGI_2 synthase [82,83] and TxA_2 synthase [84,85] have been purified to homogeneity and both enzymes appear to be related membrane-bound haemoproteins with a cytochrome *P*-450 chromophore. The levels of PGI_2 synthase and PGG/H synthase protein are co-ordinately regulated in endothelial cells by endothelial cell growth factor [74]. This finding indicates that PGI_2 synthase and perhaps other PGH_2 metabolizing enzymes are regulated in concert with PGG/H synthase.

PGE_2 synthesis requires reduced glutathione [86]. The formation of PGE_2 is catalysed by several immunologically distinct, membrane-bound PGH–PGE isomerases which lack glutathione *S*-transferase activity [87], as well as by several subtypes of soluble glutathione *S*-transferase [88]. The question of which, if any, of these enzymes is important in PGE_2 synthesis *in vivo* is unresolved. Several soluble proteins with PGH–PGD isomerase activity have also been purified [89–91]. Recently, a PGF reductase activity has been isolated from lung [92].

Synthesis of lipoxygenase products. Leukotriene and related mono-, di- and tri-hydroxy fatty acid products

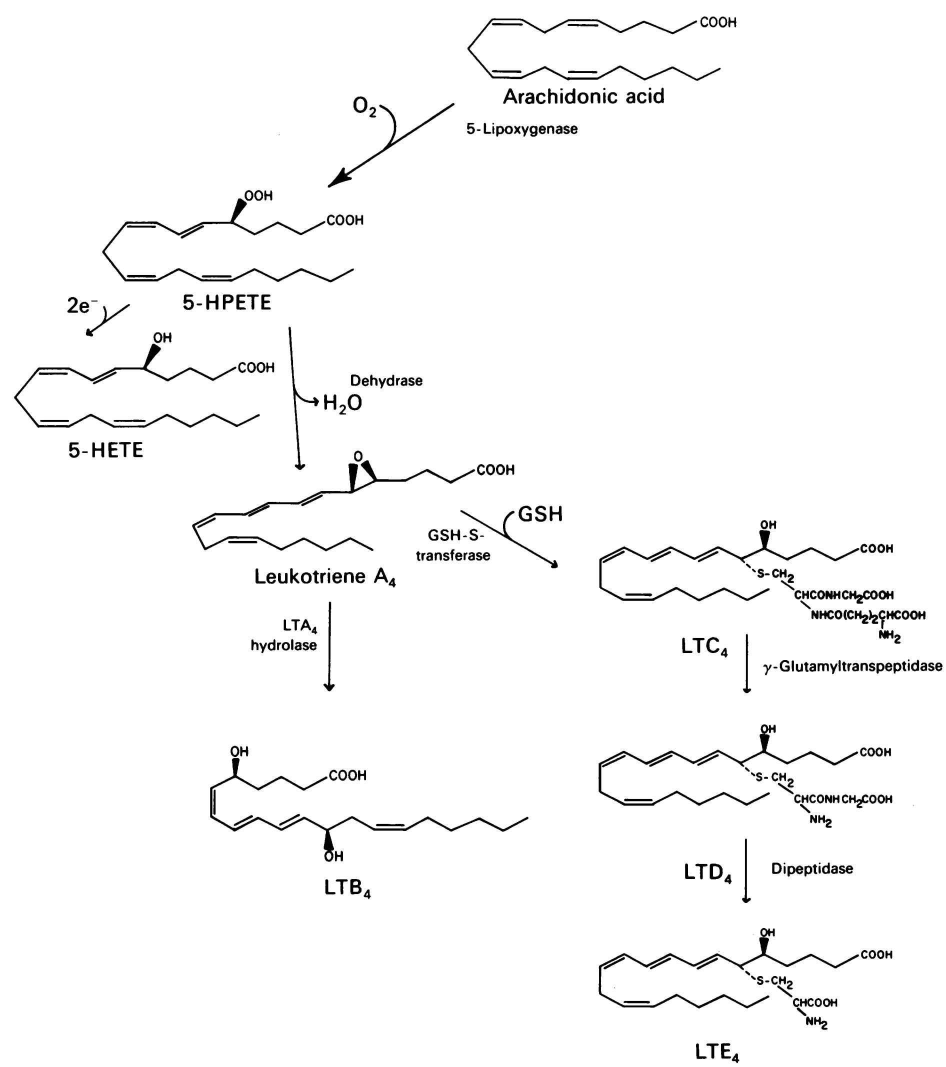

Fig. 3. The 5-lipoxygenase pathway and resulting products

are formed via lipoxygenase pathways. Samuelsson *et al.* [93] have recently provided a cogent review of this area. Briefly, there are three different mammalian lipoxygenases which catalyse the insertion of molecular oxygen into arachidonic acid at positions 5 (Fig. 3), 12 or 15. The initial product of each of these reactions is a hydroperoxyeicosatetraenoic acid (i.e. 5-, 12- or 15-HPETE) which can be reduced to the corresponding hydroxyeicosatetraenoic acid (i.e. 5-, 12-, or 15-HETE). The 12-lipoxygenase is present in platelets [94,95] and leukocytes [96], but the physiological function of 12-HETE (or 12-HPETE) is unclear. The 15-lipoxygenase is found in leukocytes [97,98] and it has been suggested that this enzyme participates in the formation of lipoxins (Fig. 1; [93]).

The 5-lipoxygenase pathway has been the lipoxygenase pathway which has received the most attention because this pathway is the one involved in leukotriene formation (Fig. 3). Thus, 5-HPETE can be converted by removal of water to an acid-labile 5,6-epoxide containing a conjugated triene structure and called leukotriene A_4 (LTA_4). Both the 5-lipoxygenase and dehydrase activities are associated with the same protein; the primary structure of this protein is now known [29,93]. LTA_4 can be converted to LTB_4 (5*S*,12*R*-diHETE) by net addition of water via the action of LTA_4 hydrolase [99,100] or to LTC_4 by addition of the glutathionyl group at C-6 by the action of a glutathione *S*-transferase [101]. Leukotrienes containing peptides or amino acids at C-6 are termed peptidoleukotrienes. LTC_4 can be cleaved by γ-glutamyltranspeptidase to produce LTD_4, and LTD_4 can be further metabolized to LTE_4 by a dipeptidase [101]. LTC_4 and LTD_4 comprise the major elements of the slow reacting substances of anaphylaxis (SRS-A); LTE_4 has about one-tenth the myogenic activity of LTC_4 or LTD_4 [93].

Mechanisms of eicosanoid actions

Eicosanoids are 'local' hormones. The concept that eicosanoids are local hormones (i.e. autocoids) originated with the studies of Ferreira & Vane [102], who demonstrated that infused PGE and PGF_α derivatives fail to survive a single pass through the circulation; this idea is now firmly supported by two general types of observations: first, that plasma concentrations of eicosanoids, except in rare situations [103,104], are less than 10^{-9} M [105–108], a concentration below which these compounds are normally unable to elicit responses;

and second, that eicosanoid synthesis is not restricted to a central endocrine organ, but rather occurs in most organs [25,35,109–111], although not necessarily in all cells comprising an organ.

The low plasma concentrations of eicosanoids are a consequence of a combination of instability, as in the case of TxA_2 ($t_{\frac{1}{2}}$ = 30 s at 37 °C; [81]), and/or active catabolism, as in the cases of PGE_2, $PGF_{2\alpha}$, PGD_2, PGI_2 [112–114], and leukotrienes [101,112,115,116]. Prostanoid catabolism begins with oxidation of the 15-hydroxyl group to yield in one step the 15-oxo-derivatives which have 10- to 100-fold less activity than the parent compounds. There are different 15-hydroxyprostaglandin dehydrogenases specific for different prostanoids. These dehydrogenases are concentrated in the lung, kidney, and placenta [112–114]. The major urinary metabolites of prostanoids are C_{16} dicarboxylic acids (i.e. dinor derivatives) which result from ω-oxidation and β-oxidation of the parent 15-oxo-13,14-dihydro catabolites [105,112]. With the leukotrienes, the first step in catabolism is hydroxylation at C-19 ($\omega-1$) or 20 (ω), which is followed by oxidation to a dicarboxylic acid and then β-oxidation [101,112,115,116].

The role of PGE_2 in water reabsorption by the kidney as a model of eicosanoid actions. One eicosanoid or another can usually be found to have a biological effect in almost every physiological setting, including, but not limited to, effects on intermediary metabolism, muscle tone and cell growth. It has been difficult to rationalize all these effects under the guise of a unifying biochemical paradigm. The two systems in which the roles of these compounds are best defined at the physiological, cellular and molecular levels are the platelet/vessel wall interaction involving prostacyclin (PGI_2) and thromboxane A_2 [117–119], and the renal water reabsorption process involving PGE_2 [120]. Of these, the water reabsorption system is the most straightforward. Accordingly, I plan to discuss the role of PGE_2 in water reabsorption in some detail as a model of how prostanoids can act (Fig. 4). I will emphasize that in the renal collecting tubule and the adjacent thick ascending limb, PGE_2 is able to operate through two different receptors — a stimulatory receptor coupled to G_s and involved in activation of adenylate cyclase (i.e. β-adrenergic-like), and an inhibitory receptor coupled to G_i and involved in inhibition of vasopressin-stimulated adenylate cyclase (α_2-adrenergic-like). Then, in completing this discussion of eicosanoid action, I will summarize the evidence which suggests that not only PGE_2, but all prostanoids and leukotrienes, operate through G protein-linked receptors.

Regulation of whole body water balance depends on drinking (input) and renal water reabsorption (input/output); the final segment of the renal tubule, the collecting tubule, is the site at which water reabsorption is regulated (Fig. 4; [121]). Water reabsorption by the collecting tubule is dependent on two factors: (*a*) the presence of circulating antidiuretic hormone (i.e. AVP) and (*b*) the existence of an osmotic gradient between the tubule lumen and the surrounding interstitium. The formation of the concentration gradient depends on the action of the ascending thick and thin limbs of Henle's loop (which together comprise the 'diluting segment') which run adjacent, but antiparallel, to the collecting tubule; the diluting segment actively pumps NaCl from the lumen into the interstitium. NaCl reabsorption by this segment is potentiated by AVP. The diluting segment itself is water impermeable, so that NaCl reabsorption by the thick limb results in the formation of both a hyper-osmotic interstitium and a hypo-osmotic tubular filtrate.

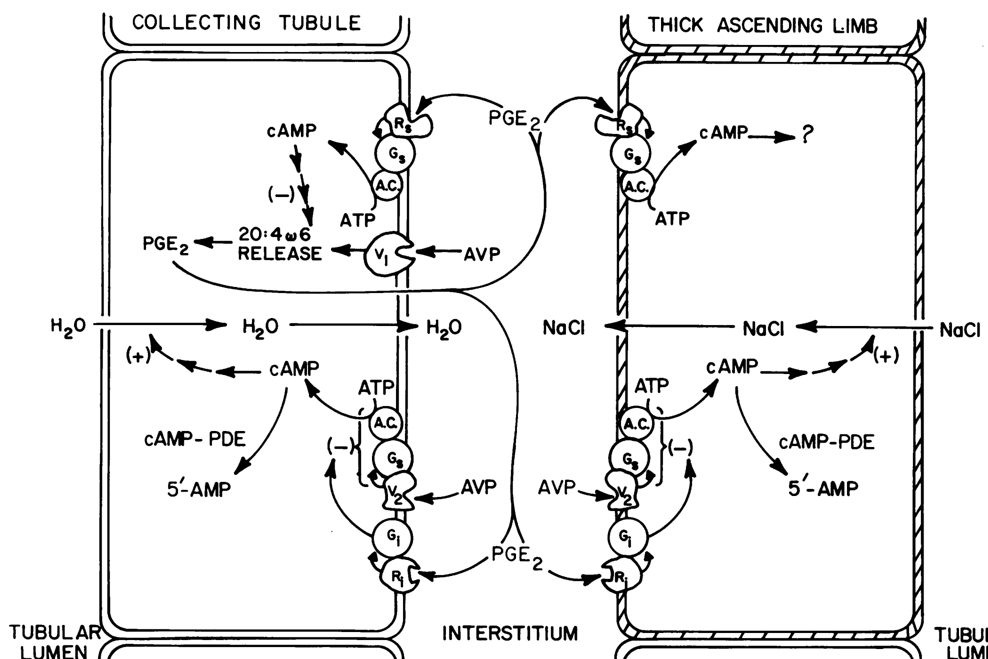

Fig. 4. Model for the regulation of NaCl and water reabsorption by PGE_2 in the thick ascending limb and collecting tubule of the kidney

Abbreviations include: R_i, inhibitory receptor; R_s, stimulatory receptor; G_s, stimulatory (cholera toxin-sensitive) guanine nucleotide regulatory (G) protein; G_i, inhibitory G protein; A.C., adenylate cyclase; V_1 and V_2, vasopressin receptors; cAMP-PDE, cAMP phosphodiesterase.

Physiological effects of PGE_2 on the collecting tubule and thick ascending limb. The effect of AVP to cause water reabsorption by the collecting tubule is mediated by cyclic AMP (Fig. 4); AVP acts via a stimulatory V_2 receptor coupled to a stimulatory guanine nucleotide regulatory protein G_s to cause cyclic AMP synthesis [122]; elevating intracellular cyclic AMP levels leads to water reabsorption; however, the mechanism by which increases in cyclic AMP are translated into the physiological response of water reabsorption are ill-defined [123,124]. By using perfused, microdissected segments of rabbit cortical collecting tubule, Grantham & Orloff showed that low concentrations of PGE_1 (10^{-9} M) inhibit the water reabsorbing (i.e. hydro-osmotic) effect of AVP [125], but that these same concentrations of PGE_1 failed to inhibit water reabsorption caused by exogenous cyclic AMP. These results suggested that 10^{-9} M PGE_1 blocked AVP-induced water reabsorption by interfering with AVP-induced cyclic AMP synthesis. Grantham & Orloff then also found a second effect of PGE_1; at higher concentrations (10^{-7} M) PGE_1, by itself, stimulated water reabsorption.

Evidence with whole animals strongly suggests that the inhibitory effect of PGE_1 (PGE_2 has the same effects) seen by Grantham & Orloff with the dissected collecting tubule is physiological. Experimental manoeuvres such as indomethacin treatment [126,127] or essential fatty acid deficiency [128] which cause decreased renal prostaglandin synthesis result in the formation of a hyperosmotic urine, as would be expected if there were diminished inhibitory control by prostaglandins of the water-reabsorbing effect of AVP. The observations which indicate that PGE_2 is actually available *in vivo* to regulate water reabsorption are: (*a*) that the collecting tubule is one of only two tubular regions where prostaglandins are synthesized [129–131] and (*b*) that the major prostanoid synthesized by the collecting tubule is PGE_2 [43,132].

AVP can stimulate NaCl reabsorption in the thick ascending limb of Henle's loop, and cyclic AMP also appears to be the second messenger which mediates this effect (Fig. 4; [133–135]). Moreover, AVP-induced NaCl reabsorption in the perfused mouse thick limb is attenuated by PGE_2 via a pertussis toxin-sensitive mechanism [135]. These results suggested that the immediate biochemical effects of AVP and PGE_2 on cyclic AMP metabolism in both the collecting tubule and the thick limb are analogous, although the physiological endpoints — water flow and NaCl reabsorption, respectively — are different.

Cellular effects of PGE_2 on the collecting tubule and thick ascending limb. Studies with perfused tubule preparations suggested that PGE derivatives would inhibit AVP-induced cyclic AMP synthesis by both the collecting tubule and the thick limb. This model has now been tested with purified preparations of both collecting tubule cells and thick limb cells [136–138]. Isolated collecting tubule cells were found to synthesize cyclic AMP in response to AVP, and low concentrations of PGE_2 ($\leqslant 10^{-8}$ M) were found to inhibit AVP-induced cyclic AMP accumulation by intact cells (Fig. 4; [137]). The inhibitory effect of PGE_2 was blocked by pretreatment of the cells with pertussis toxin, suggesting a role for an inhibitory guanine nucleotide regulatory protein such as G_i. Moreover, PGE_2 was found to inhibit AVP-induced adenylate cyclase activity directly in membranes prepared from freshly isolated rabbit cortical collecting tubule cells [137]. Virtually identical results have been obtained with thick limb cells [138]. These inhibitory responses to PGE_2, which are analogous to α_2-adrenergic receptor mediated responses of the human platelet to adrenaline, suggested that PGE_2 can operate through an inhibitory receptor coupled to G_i to inhibit hormone-stimulated adenylate cyclase in both the collecting tubule and the thick limb.

Higher concentrations of PGE_2 ($\geqslant 10^{-7}$ M) caused stimulation of the adenylate cyclase activity in both collecting tubule and thick limb cells [137,138]. Thus, there is a second PGE response presumably mediated through G_s. In the case of the collecting tubule, the increase in cyclic AMP formation induced by PGE_2 would explain the observation of Grantham & Orloff [125] that high concentrations of PGE_1 stimulate, rather than inhibit water reabsorption.

PGE receptors of collecting tubule and thick limb cells. The inhibitory and stimulatory responses to PGE of collecting tubule and thick limb cells suggest that each cell type possesses two types of PGE receptors, an inhibitory receptor coupled to G_i and a stimulatory receptor coupled to G_s.

There is a [^{3}H]PGE_2 binding activity with properties expected for an inhibitory, G_i-linked PGE receptor associated with membranes from the outer medulla of canine and rabbit kidney [139] and purified collecting tubule [140] and thick limb cells [138]. The K_D for PGE_2 binding to membranes is about 10 nM, a concentration at which PGE_2 causes half-maximal inhibition of AVP-stimulated cyclic AMP formation [137]. Addition of guanine di- and trinucleotides, but not GMP or adenine nucleotides, causes a highly unusual increase in binding affinity of about 2-fold with no apparent change in $B_{max.}$, and this effect of guanine nucleotides on binding is eliminated by treatment of the membranes with pertussis toxin. Substitution at the methyl end of the PGE_2 molecule (e.g. 16-phenoxy or 17-phenyl groups) has little effect either on binding or on the ability of PGE_2 to inhibit AVP-induced cyclic AMP formation. In fact, sulprostone (16-phenoxy-17,18,19,20-tetranor-PGE_2 methylsulphonilamide) is equipotent to PGE_2 in inhibiting [^{3}H]PGE_2 binding to membranes prepared from the collecting tubule [140] and in causing inhibition of AVP-induced cyclic AMP formation by rabbit cortical collecting tubule cells [137]. As will be discussed next, these pharmacological properties are quite different from those characteristic of PGE-induced cyclic AMP formation. Interestingly, the G_i-linked PGE receptor of the collecting tubule and thick limb can be solubilized from the renal medulla with digitonin. This solubilized receptor occurs in a complex with G_i and exhibits PGE_2 binding properties very similar to those of the membrane-bound receptor [139]; these observations indicate that the coupling of this inhibitory PGE receptor to G_i is direct.

Although PGE_2, when used at relatively low concentrations ($\leqslant 10^{-8}$ M), will inhibit AVP-induced cyclic AMP formation by collecting tubule and thick limb cells, higher concentrations of PGE_2 actually cause stimulation of cyclic AMP formation [137]. This effect is specific for E series prostanoids, and suggests that there is a stimulatory PGE receptor associated with the collecting tubule and thick limb. Pharmacological

Table 1. Prostanoid and leukotriene receptors coupled to G proteins

Receptor/ G protein	Evidence for G protein coupling	Tissue	Reference
PGE/G_i	GTP inhibits PGE binding and pertussis toxin eliminates GTP effect	Renal medulla, adipocyte	[139,148]
PGE/G_2	GTP inhibits PGE binding; PGE binding correlates with adenylate cyclase activation	Frog erythrocyte	[149]
$PGE/G_?$	GTP inhibits PGE binding; G protein not identified	Cerebral cortex	[150]
PGE/G_{am}	PGE stimulates GTPase activity	Adrenal medulla	[151]
$PGF_\alpha/G_?$	GTP inhibits $PGF_{2\alpha}$ binding	Renal cortex	W. K. Sonnenburg & W. L. Smith, unpublished work
$PGI(PGE_1)/G_s$	PGE_1 stimulates GTPase; radiation inactivation indicates receptor associated with a G protein	Platelets	[153,154]
$TxA/PGH/G_?$	PGH analogues stimulate GTPase activity	Platelets	[155,156]
$LTD_4/G_?$	GTP inhibits LTD_4 binding	Lung	[157]

evidence suggests that the stimulatory PGE receptor is distinct from the inhibitory receptor and that the stimulatory PGE receptor is coupled to a G protein, presumably a G_s. For example, sulprostone fails to stimulate cyclic AMP formation even at 10^{-5} M, a concentration at which PGE_2 causes maximal stimulation of cyclic AMP synthesis [137]. Thus, sulprostone, which is a potent agonist of the inhibitory PGE receptor, apparently does not interact with the stimulatory PGE receptor. When collecting tubule cells are cultured for several days, the inhibitory but not the stimulatory response to PGE_2 is lost [137,140]; the affinity for PGE_2 of membranes from cultured cells, which exhibit only a stimulatory response to PGE_2, is lower than that seen with membranes from fresh cells, which exhibit both stimulatory and inhibitory responses. Furthermore, GTPγS inhibits the binding of [^{3}H]PGE_2 to membranes from cultured cells, whereas GTPγS stimulates binding to membranes from fresh cells (via its effect on binding of PGE to the inhibitory receptor; [140]).

In summary then, there is evidence for two different PGE receptors in the renal collecting tubule, one coupled to G_i and one coupled to G_s. The major physiological effect of PGE_2, which is formed by the collecting tubule in response to AVP (Fig. 4; [42–44]), is to act via G_i-linked PGE receptors on both collecting tubule and thick limb cells, thereby serving as a biochemical governor to attenuate both water-reabsorbing and NaCl-reabsorbing responses to AVP. In short, PGE_2 serves as an intercellular local hormone regulating a response to a circulating hormone (AVP) and serving to co-ordinate the actions of two cell types (the collecting tubule and thick limb).

The function of PGE_2, acting via the stimulatory PGE receptor in the collecting tubule, is unclear. One can speculate that when the collecting tubule is exposed to very high concentrations of AVP, maximal PGE_2 synthesis occurs, and that the resulting PGE_2, acting via the stimulatory receptor, may transiently augment water reabsorption; however, large increases in cyclic AMP levels in the collecting tubule are known to attenuate PGE_2 production, probably by blocking arachidonate release [141]. Thus, the stimulatory PGE receptor may be part of a feedback mechanism preventing excessive PGE_2 formation by the collecting tubule (Fig. 4). In addition (or alternatively), supramaximal increases in cellular cyclic AMP occurring when collecting tubule or thick limb cells are exposed to high concentrations of both AVP and PGE_2 may activate cyclic AMP-dependent receptor kinase activities leading to down-regulation of responses to both agonists [142].

Eicosanoids act via G protein-linked receptors. A major lesson from studies on the effects of PGE on the renal collecting tubule is that prostanoids can act via receptors which, analogous to adrenergic receptors, are directly coupled to guanine nucleotide regulatory proteins. The question that then arises is whether all eicosanoid actions can be explained as occurring through receptors which are G protein-linked. Since no eicosanoid receptors have been purified to homogeneity for appropriate reconstitution studies with isolated G proteins, the question cannot be answered directly. However, the answer is probably yes, and the reasoning is as follows. An

Fig. 5. General biochemical mechanism of eicosanoid actions

Eicosanoids interact with specific receptors coupled to G proteins. Receptor occupancy in the presence of GTP causes dissociation of the G protein into an active α-GTP transducer form which then interacts with an effector (e.g. adenylate cyclase) to cause changes in the level of a second messenger or intracellular ion.

eicosanoid receptor can be considered to be G protein-linked if eicosanoid binding is affected by GTP derivatives and/or if addition of the eicosanoid to a plasma membrane preparation stimulates GTPase activity [143]. Based on either criterion, receptors (i.e. binding activities) for eicosanoids which have been tested for these properties qualify (Table 1).

A two-part summary statement about eicosanoid actions then is that: (*a*) prostanoids and leukotrienes are local hormones functioning to co-ordinate effects of those other hormones which induce eicosanoid synthesis, and (*b*) eicosanoids function through G protein-linked receptors to elicit their cellular effects (Fig. 5). Different trimeric G proteins, when activated by interaction with appropriate, ligand-occupied receptors, are known to bring about changes in the concentrations of second messengers (cyclic AMP, InsP_3, InsP_4, diacylglycerol or Ca^{2+}) or intracellular ions (K^+ or, perhaps, Na^+ and H^+) by stimulating adenylate cyclase, inhibiting adenylate cyclase, activating phospholipase C, opening or closing Ca^{2+} or K^+ channels, or possibly promoting Na^+/H^+ exchange [144]. The multitude of biological effects produced by eicosanoids can be understood if there are several types of receptors specific for each eicosanoid, with each receptor coupled to a different G protein mediating a different biochemical event. There may even be subtypes of eicosanoid receptors coupled to the same G protein, as appears to be the case with β- and α_2-adrenergic receptors [145–147].

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