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CYTOCHROME P-450 METABOLISM OF ARACHIDONIC ACID

John C. McGiff

Department of Pharmacology, New York Medical College, Valhalla, New York 10595

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INTRODUCTION

The generation of biological mediators from arachidonic acid (AA) results from oxygenation reactions that are regiospecific and stereospecific and involve three major enzyme systems: cyclooxygenase, lipoxygenases, and cytochrome P-450-dependent monooxygenases (1). The last named has been designated the third pathway of AA metabolism (2). Cytochrome P-450-dependent monooxygenases that metabolize AA have a much wider distribution than anticipated from our initial studies on the medullary thick ascending limb of the loop of Henle (mTALH) (3). For example, cytochrome P-450-dependent AA (P-450-AA) metabolism predominates in the corneal epithelium that exhibits transport characteristics similar to those of mTALH: Na^+, K^+ and Cl^- cotransport inhibitable by furosemide (4, 5). This pathway of AA metabolism is also represented in the vasculature where cytochrome P-450 monooxygenase activity is stratified, the highest activity being present in the intima (6).

These AA products are endowed with biological properties—vasoactivity and the ability to affect ion movement—that identify them as potentially important to the regulation of the circulation and extracellular fluid volume and composition (7). Although the focus of this review is AA metabolism by cytochrome P-450-dependent monooxygenases, it should be recalled that the biological role of the cytochrome P-450 system includes metabolism of cholesterol, bile acids, vitamins, and steroids in addition to xenobiotics and drugs (8). Further, thromboxane and prostacyclin synthases are cytochrome

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P-450-like enzymes (9, 10). Thus, experimental interventions aimed at manipulating cytochrome P-450-dependent AA metabolism may also affect formation of mineralocorticoids, thromboxane, and prostacyclin. This may complicate interpretation of the effects of an intervention directed at modifying synthesis of AA metabolites by cytochrome P-450-dependent monooxygenases.

The seminal studies of Capdevila, Falck, and their associates (11, 12), as well as Oliw, Guengerich & Oates (13), and Morrison & Pascoe (14), should be acknowledged as prime movers in this area of research. Two recent reviews are recommended to the interested reader (15, 16).

AA METABOLISM BY CYTOCHROME P-450 MONOOXYGENASES

Cytochrome P-450-dependent metabolism of AA requires molecular oxygen and NADPH in a 1:1 stoichiometry. Other polyunsaturated fatty acids may substitute for AA as substrate in cytochrome P-450-dependent reactions (16). The substrate specificity requirements for metabolism by this system are not so narrowly defined as for cyclooxygenase and lipoxygenases. Variations in substrate preference by P-450 isozymes relative to metabolism of AA have been observed (17). The cytochrome P-450 system catalyzes three types of oxidative reactions involving AA (Figure 1).

1. Epoxidation of AA gives rise to four epoxides: 5, 6; 8, 9; 11, 12, and 14, 15 epoxyeicosatrienoic acids (EETs). These, in turn, can be converted by

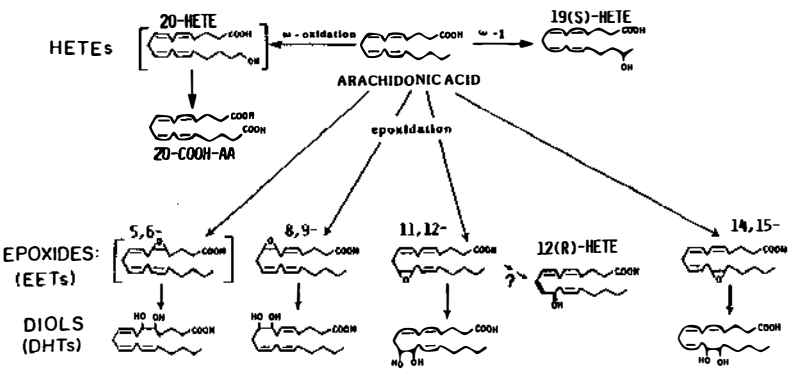

Figure 1 Arachidonic acid (AA) metabolism by cytochrome P-450-dependent monooxygenase to ω and ω-1 hydroxyeicosatetraenoic acids (HETEs), epoxyeicosatrienoic acids (epoxides/EETs), and dihydroxyeicosatetraenoic acids (diols/DHTs). Not shown are 8-,9-,11-, and 15-HETEs, products of allylic oxidation. The "brackets" indicate those products that can be transformed by cyclooxygenase. The origin of 12(R)-HETE is uncertain; therefore, the question mark.

epoxide hydrolases to the corresponding vicinal diols, the dihydroxyeicosatrienoic acids (DHTs) (13). The 5,6-EET appear to require an additional step, transformation by cyclooxygenase, for expression of vasodilator activity in most vascular beds (18).

2. Allylic oxidation forms hydroxyeicosatetraenoic acids (HETEs). Hepatic microsomal cytochrome P-450 produced six regioisomers without stereoselectivity: 5-, 8-, 9-, 11-, 12- and 15-HETEs (12). 12(R)-HETE has commanded attention because of its biological profile: inhibition of Na^+-K^+-ATPase (19) and renin release (20) and possible function as a vascular hormone involved in tissue responses to injury (21).
3. Omega (ω) and ω-1 hydroxylation of AA generates 20- and 19-HETEs, respectively. These eicosanoids have prohypertensive properties and have been implicated in the development of hypertension in the spontaneously hypertensive rat (SHR) (22).

EETs are esterified to the *Sn*-2 position of glycerolipids (23). This capability assumes functional significance relative to: (*a*) Membrane characteristics as measured by changes in permeability and activity of membrane-bound enzymes, and (*b*) EETs acting as intracellular or intercellular messengers after release from phospholipids and thereby participating in transmembrane signaling. Heretofore, eicosanoids were considered to act as intercellular messengers and to be synthesized on demand only. For example, prostaglandin production is very low in resting tissues; however, levels can be increased by as much as several hundredfold in extracellular fluid within one to two minutes in response to appropriate stimuli, including hormones, neurotransmitters, and tissue injury (24). Synthesis of prostanoids is thought to result in their immediate extrusion into the extracellular space where they act on receptors in the plasma membrane. Recent evidence is compatible with an intracellular role for some AA metabolites. Eicosanoids generated by a non-cyclooxygenase pathway, by either a cytochrome P-450 monooxygenase or a lipoxygenase, may modulate the effects of hormones/neurotransmitters on ion channels by acting within the cell of origin (25).

BIOCHEMISTRY OF CYTOCHROME P-450 OXYGENATION OF AA

The hepatic cytochrome P-450 system accounts for 4–5% of rat hepatic microsomal protein, of which cytochrome P-450 epoxygenases constitute major components as indicated by concentrations of EETs in rat liver of ca 1μg/g wet tissue (26), a value similar to that found in the rat kidney (27). As noted, EETs may be esterified to the *Sn*-2 position of cellular glycerolipids including phosphatidylethanolamine and phosphatidylinositol.

EETs represented two-thirds, and HETEs and 19- 20-HETEs almost one-third, of AA products generated by microsomes obtained from the liver of

adult male rats not treated with enzyme inducers (17). The smallest component of P-450-AA products formed by hepatic microsomes, accounting for ca 7% of the total, has been identified as a mixture of monohydroxylated (16-, 17-, and 18-) eicosatetraenoic acids (28). Within the kidney regional variations in P-450-AA metabolites have been reported by Takahashi et al (27) based on the biosynthetic profile of cortical vs medullary microsomal fractions. EETs are the principal P-450-AA products of the renal cortex of the rat, where they account for ca 60% of total P-450-AA products, whereas in the medulla, 19- and 20- HETEs represent 90% of P-450-AA metabolites generated by microsomal fractions. These findings on P-450-AA product formation by the rat kidney differ from those reported by Schwartzman et al (29) for the rabbit kidney, in which species large differences in ω and ω-1 production of metabolites, when comparing cortex vs medulla, were not observed. The specific activity of P-450-AA metabolism is higher in the kidney than liver of the rabbit, with the highest activity, threefold that of the liver, in the outer medulla. The pattern of cytochrome P-450-dependent AA metabolism in the human kidney is the same as reported for the rat and rabbit kidney (30).

The production of HETEs by hepatic microsomes can be increased by treatment with β-naphthoflavone (β-NF), which has the additional effects of reducing formation of EETs while enhancing that of 19- and 20-HETEs (28). In a related study, Oliw has reported conversion of AA to 18(R)-HETE by seminal vesicular microsomes obtained from the cynomolgus monkey (31). The biological activities of the 16-, 17-, and 18-HETEs are unknown except for a preliminary report on contraction of lung strips and relaxation of isolated arteries of the guinea pig by 18(R)-HETE (31). If these HETEs approach, in terms of important biological activities, those of 19- and 20-HETEs, then these newly identified AA products will assume possible functional significance, moving them from the realm of biochemical curiosities to that of putative components of physiological systems. This attempt to place novel AA products in a physiological or pathological context has been a principal objective of our studies and is abundantly demonstrated in the parallel efforts directed towards the biochemical and biological-functional characterization of P-450-AA products generated by rabbit mTALH (3) and by the kidneys of hypertensive rats (22) and rabbits (2).

Epoxidation of AA in man is catalyzed by a distinct hepatic cytochrome P-450 isozyme that Schwartzman et al (32) purified to electrophoretic homogeneity. In a reconstituted system the hepatic P-450 isozyme oxidized AA in a nonpreferential manner into the four regioisomeric EETs: 5,6-; 8,9-; 11,12-; and 14,15-EET. Regiospecific formation of EETs by rat hepatic microsomes as well as in reconstituted hepatic enzyme systems is inducible by phenobarbital treatment, resulting in generation of EETs of high optical purity (17, 33). The demonstration of stereospecificity of AA product formation is important as these AA products can arise nonenzymatically by autooxidation

of AA, yielding racemic EETs. Brash has stated the problem succinctly: "Autooxidation is a facile process which is difficult to eliminate. The addition of high concentrations of polyunsaturated fatty acid substrates to incubations of cells will certainly lead to the detection of non-enzymetically formed hydroperoxyeicosatetraenoic acids [H(P)ETEs] if the analytic methodology is sufficiently sensitive" (34). The stereoselective formation of an EET is a "decisive criterion" for their enzymic origin (26). Stereoisomers usually differ substantially in their biological properties; for example, the principal AA product of the cytochrome P-450 system in the cornea, 12(R)-HETE, unlike its enantiomer 12(S)-HETE, inhibits Na^+-K^+-ATPase activity (19). Moreover, despite apparent similarities in some of the biological actions of 12(R)- and 12(S)- HETE, such as inhibition of renin release, closer examination discloses important temporal differences, notably in the onset and duration of their inhibitory effects on renin release (20).

The principal product of hepatic microsomal epoxygenase was 11,12-EET of which greater than 80% was the 11(R)-, 12(S)- enantiomer, indicating stereospecificity of AA product formation (17). The 11,12-epoxide has also been reported to be the principal P-450-AA metabolite generated by rat (27) and rabbit renal cortical microsomes (29). Cytochrome P-450 enzyme induction with phenobarbital resulted in increased optical purity of the EETs formed by the liver and altered the stereoselectivity of microsomal epoxygenases favoring the formation of 11(S)-, 12(R)- rather than 11(R)-, 12(S)-EET, the principal product before administration of phenobarbital (17). This inversion of the principal enantiomer of 11,12-EET generated by hepatic microsomes in response to phenobarbital was also noted for the other EETs. In addition, phenobarbital increased synthesis of total EETs by hepatic microsomal enzymes. The effects of phenobarbital on production of stereoselective EETs had different time courses for each of the EETs, i.e. stereoselectivity of 14,15-EET was achieved after one day of treatment whereas two to three days were required for demonstrating alterations in stereoselective formation of 8,9- and 11,12-EETs. Changes in stereoselectivity of epoxygenases induced by phenobarbital stand in contrast to stereoselectivity of the cyclooxygenase and lipoxygenase pathways of AA metabolism that exhibit unalterable stereospecific product formation.

THE CYTOCHROME P-450 SYSTEM IN THE NEPHRON

We entered the field by misadventure, as Princes of Serendip, when after isolation of cells from the mTALH we, initially and incorrectly, identified a lipoxygenase as the major pathway of AA metabolism (3). In this segment of the nephron, the mTALH, cyclooxygenase activity was negligible, if not absent, which challenges concepts based upon the ubiquity of the prostaglandin-synthesizing capacity of cells. Our findings and those of others, when

viewed in a larger context, have forced several conclusions of a more general nature that can serve as directional markers for future studies on P-450-AA metabolites:

1. The capacity of cells to synthesize prostaglandins varies greatly and was first described by Smith & Bell (35) for the nephron, where cyclooxygenase is distributed in a discontinuous fashion. It is concentrated in the collecting tubules and "skips" segments including the mTALH. Based on conventional methods, it is difficult to eliminate with certainty a small but potentially significant ability to synthesize prostaglandins by a given nephron segment. Metabolism of AA by cyclooxygenase in the nephron appears to be discretely localized and is overshadowed in some segments by a considerable capacity to generate cytochrome P-450-dependent AA metabolites, e.g. in the mTALH (3) and, as has been recently reported, in the S_1 segment of the proximal tubules (36). The cortical collecting ducts, perhaps, occupy an intermediate position as they possess a considerable prostaglandin biosynthetic capacity and are also able to synthesize EETs (37).
2. The metabolism and expression of a particular biological action of several eicosanoids require the cooperative interaction of different cell types because of the importance of transcellular metabolism to the generation of these AA metabolites. For example, in addition to thromboxane, prostaglandin endoperoxides and 12(S)-HETE are formed by platelets (38) and may undergo transformation in blood vessels and white cells, PGH_2 by endothelial cells to form prostacyclin (39) and 12(S)-HETE by neutrophils to form 12, 20-HETE (40). These products differ in their biological properties from the platelet precursors. In like manner, transcellular metabolism via cyclooxygenase of two recently described eicosanoids, 20-HETE and 5,6-EET synthesized in the mTALH and S_1 segment of the proximal tubules, respectively, by cytochrome P-450 oxygenases, is crucial to the expression of their vasoactivity on most blood vessels (18, 41: Figure 2).

   A reverse sequence has also been observed. A cyclooxygenase product can be metabolized by a cytochrome P-450-dependent monooxygenase, a step that results in the generation of an AA product possessing different properties from its precursor. The early reports on prostaglandin metabolism by the P-450 system addressed ω-1 and ω-hydroxylation of the stable prostaglandins, PGE_2 and $PGF_{2\alpha}$, but provided little information on the acquisition by the metabolite of a different spectrum of biological properties (42). This omission has marred other reports on cytochrome P-450-dependent metabolism of prostaglandins such as conversion of PGI_2 to the 5,6 epoxide (43) or the reverse sequence in which 5,6 epoxidation precedes the generation of prostaglandin analogs of 5,6-EET by cyclooxy-

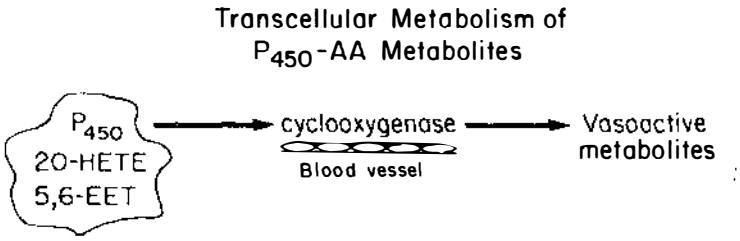

Figure 2 The cellular proximity of the cytochrome P-450 monooxygenase to certain segments of the renal tubules and cyclooxygenase of several structures including interstitial cells and blood vessels may confer a unique ability on AA metabolites to affect renal tubular transport in the cell of origin and then be transformed to vasoactive mediators by cyclooxygenase. This possible sequence is shown in the figure: either 20-HETE or 5,6-EET arising from the P-450 system of the tubules on extrusion into the interstitium may be converted to vasoactive products by the cyclooxygenase of adjacent structures such as blood vessels.

genase (44). A metabolic sequence, operating through enzymes acting in different but contiguous cells within a renal zone or tubular segment, and, presumably, also in extrarenal tissues such as blood vessels, provides a mechanism for eicosanoids to alter tissue function selectively. For example, an eicosanoid arising from the cytochrome P-450 system in a nephron segment may affect transport function in that segment and after extrusion into the extracellular space, can modify regional blood supply subsequent to its conversion by vascular cyclooxygenase (Figure 2). Either 5,6-EET, or 20-HETE, acting via such a sequence, may link metabolic activity and/or transport function of a tissue to local blood flow (see below).

3. Initially, the biological properties of the AA metabolites should be defined broadly as, for example, their effects on vascular tone and Na^+-K^+-ATPase activity (3, 45). Characterization of the more easily defined biological properties of P-450-AA metabolites can proceed simultaneously with establishing optimal conditions for generating AA metabolites by a cell type (3) or by microsomes obtained from a renal zone (cortex, outer and inner medulla) (29) or a specialized tissue such as the cornea (4). The progression of our studies was facilitated by biological characterization of the P-450-AA products as a first step, one antecedent to structural analysis of novel AA metabolites that was followed by their chemical synthesis. The last (J. R. Falck, personal communication) was essential to the in-depth examination of the biological activity of P-450-AA metabolites and verification of the structural analysis of these metabolites recovered from HPLC fractions. Thus, comparison of the biological activity of authentic standards of P-450-AA metabolites with that of material in fractions recovered after HPLC separation determines the degree of identity/congruity of endogenous material and authentic standards of the

principal P-450-AA products. This step, biological profiling, has the additional advantage of bypassing the need for obtaining relatively large quantities of AA metabolites from cells of limited availability required to establish the stereochemistry of novel eicosanoids. This strategy was used in the identification of cytochrome P-450-dependent AA metabolites synthesized by the corneal epithelium (19). One of the AA products generated by corneal microsomes inhibited Na^+-K^+-ATPase. Mass spectrometric analysis indicated it to be 12-HETE (R. C. Murphy, personal communication). However, the amount of the eicosanoid generated by corneal microsomes fell short of the requirements for stereochemical analysis. Nonetheless, it was possible to establish the stereochemistry of the eicosanoid with confidence by comparing the biological properties of authentic 12(S)- and 12(R)-HETE to those of the endogenous eicosanoid. Inhibition of Na^+-K^+-ATPase by 12(R)-HETE demonstrated a potency and time course of activity indistinguishable from that of the endogenous eicosanoid recovered from the HPLC peak associated with Na^+-K^+-ATPase inhibitory activity (19).

The availability of 12-HETE stereoisomers also permitted an examination of their individual effects on renal function, a study of some significance, as a 12-HETE was reported to be a principal product of AA metabolism in isolated glomeruli and in renal cortical homogenates (46). Major differences were shown on comparing the effects of 12(S)-HETE and 12(R)-HETE on renal function: 12(R)-HETE had a prolonged natriuretic effect and inhibited renin release for an extended period whereas 12(S)-HETE exhibited only a transient action on sodium excretion and renin release (20). Further, only 12(R)-HETE prevented the decline in GFR, characteristic of the rat-isolated kidney, suggesting a glomerulotrophic action of 12(R)-HETE.

4. The flux of AA through P-450-dependent monooxygenases can be controlled by manipulating the level of cytochrome P-450 (29). It is, therefore, possible to relate changes in P-450-AA metabolite formation to functional alterations as has been done for vasomotion (47) and the activity of several transporting epithelia such as the mTALH (48) and cornea. The experimental basis for this had been established by Schwartzman et al (29). They demonstrated that induction of renal cytochrome P-450 by 3-methyl cholanthrene (3MC) and β-NF increased cytochrome P-450 content and enhanced simultaneously arylhydrocarbonhydroxylase (AHH) activity, an index of P-450-dependent monooxygenase activity, in both the renal cortex and medulla. These changes correlated with increased formation of P-450-AA metabolites by renal microsomes. In contrast, cobalt ($CoCl_2$)-induction of heme oxygenase, a heme-degradative enzyme, reduces the availability of heme for hemoproteins such as cytochrome P-450 monooxygenase, resulting in decreased AA metabolism via the third

pathway. This study confirmed the close relationship that exists intrarenally between the cytochrome P-450 system and metabolism of AA. As corresponding changes in synthesis of P-450-AA metabolites were produced in intact mTALH cells by these interventions, Escalante et al were able to relate altered transport function to changes in P-450-AA metabolism in this segment of the nephron (see below) (48).

Qualitative and quantitative alterations in P-450-AA metabolism in mTALH can be produced by modifying salt intake or by renal ischemia (49, 50). Carroll et al have shown that formation of vasodilator P-450-AA products can be effected selectively by potassium-loading rabbits for two days (51). In contrast, production of renal ischemia by constricting the aorta caused an increase in all P-450-AA metabolites generated by mTALH cells (50). The P-450-AA metabolites generated by mTALH in response to renal ischemia act in a defensive capacity to limit the degree of mTALH cell injury by reducing energy-dependent Na^+-K^+-ATPase activity and by producing local vasodilatation in this renal zone that has been described as operating "on the verge of anoxia". Carroll has suggested that increased formation of P-450-AA metabolites thereby contribute to energy conservation in this nephron segment by: (*a*) inhibiting Na^+-K^+-ATPase, a major determinant of energy expenditure in the mTALH, and (*b*) reducing the chemical gradient between tubular fluid and the medullary interstitium by increasing medullary blood flow (50).

5. Cytochrome P-450 related monooxygenase activity within the kidney can be stimulated by treatment with either glucocorticoids or mineralocorticoids, apparently via a mechanism involving adenylate cyclase (45, 52). The evidence for this linkage is based on several related studies: First, the capacity of cortical and medullary microsomes obtained from adrenalectomized rabbits to generate P-450 AA products, particularly 19- and 20-HETEs, was greatly reduced by adrenalectomy and was associated with negative sodium balance (53). Administration of a mineralocorticoid, deoxycorticosterone acetate (DOCA), restored the capacity of the kidney to generate P-450-AA metabolites and produced positive sodium balance (53), the latter presumably reflecting increased synthesis of vasoconstrictor and salt-retaining eicosanoids, 20- and 19-HETEs (22). As adrenalectomy impairs hormonal responsiveness of adenylate cyclase within nephron segments to cholera toxin but not to forskolin, these results are compatible with actions of adrenal steroids on G_s proteins (52). Second, formation of P-450-AA products by the nephron is stimulated by interventions that increased cyclic-AMP, thereby linking the adenylate cyclase and P-450 systems (45). Thus, cytochrome P-450-AA metabolism by mTALH cells can be stimulated by either AVP or calcitonin via an adenylate cyclase-related mechanism, as the hormonal effects on formation of P-450-AA products are increased by phosphodiesterase inhibition

and mimicked by analogs of cyclic-AMP (45). Two segments of the nephron, which have been demonstrated to form P-450-AA metabolites, have been identified as responding to adrenal steroids selectively: the principal cells of the collecting tubules to mineralocorticoids, and the mTALH to glucocorticoids (52). In each nephron site, the adrenal steroid controls the adenylate cyclase system at the level of G_s protein, presumably by affecting the GTP-binding protein that couples the hormone receptor to the catalytic unit of adenylate cyclase, thereby determining responsiveness of the TALH to AVP, glucagon, and calcitonin, and the collecting tubules to AVP (52). An adenylate cyclase-dependent mechanism that regulates expression of cytochrome-P-450 isozymes has been described in the adrenal gland (54).

Another important consideration relative to the action of adrenal steroids on renal function, and linked to changes in AA metabolism, is related to effects on GFR and renal blood flow (55). Glucocorticoids increase GFR and alter renal vascular responsiveness to prostaglandins, probably by affecting both cyclooxygenase and cytochrome P-450-dependent AA metabolism as well as by altering "the expression of receptor-mediated contractile responses to eicosanoids" (55, 56). Sessa & Nasjletti (56) have shown selective attenuation of renal vasoconstrictor responses to U46619, the thromboxane/prostaglandin endoperoxide mimetic, and to $PGF_2\alpha$ after dexamethasone treatment of rabbits and have extended these findings to vasodilator prostanoids (55). Prostacyclin and PGE_2-induced renal vasodilatation were attenuated and converted to vasoconstriction, respectively, when rabbits were treated with dexamethasone. These effects of the glucocorticoid can be explained by induction of P-450 monooxygenase activity that results in transformation of prostanoids to ω, ω-1, and ω-2 hydroxylated products and possibly epoxides. Modification of the biological activity of prostaglandins and other eicosanoids by the P-450 system appears to be an important mechanism that thus far has been neglected. Further, it assumes physiological significance when viewed in terms of a cytochrome P-450 isozyme activated by changes in hormonal status and having the capacity to transform prostaglandins. For example, a greater than 100-fold induction of a pulmonary cytochrome P-450 isozyme, prostaglandin ω hydroxylase, has been observed in pregnant rabbits (8). The low K_m for prostaglandin substrates, regioselectivity, and remarkable capacity for induction during pregnancy mark this P-450 isozyme as physiologically important (8).

THE VASCULAR CYTOCHROME P-450 SYSTEM

Within the vasculature, the cytochrome P-450-dependent oxygenase is localized primarily in the endothelium (6), analogous to the segmental localization

of the cytochrome P-450 pathway of AA metabolism within the nephron (57). This conclusion is based on several lines of evidence:

1. The cytochrome P-450-dependent vasodilator response to AA was greatly diminished after mechanical or chemical deendothelialization (58). These functional studies are in accord with those based on immunofluorescent (59) and biochemical methods (6) localizing cytochrome P-450-AA metabolism mainly to the endothelium.
2. Cultured endothelial cells can metabolize AA via cytochrome-P-450 monooxygenases, as evidenced by the recovery of EETs and HETEs (60, 61). For endothelial cells originating from either different sites or species, the cultured cells frequently differ in the principal P-450-AA products formed. Moreover, cultured cells generate P-450-AA products that may differ from the same cells when freshly isolated (62).
3. Within the aorta, the activity of a cytochrome P-450-related hydroxylase (AHH) was several fold greater in the intima than in the media (6). The demonstration of cytochrome P-450 enzyme activity within a tissue, however, does not allow the conclusion that the tissue possesses the capacity to metabolize AA via a P-450 pathway. Certain cytochrome P-450 isozymes are unable to metabolize AA (17). Therefore, it is necessary to demonstrate that P-450-AA products can be generated by a particular vascular bed to establish a possible role for P-450-AA metabolites of vascular origin in that tissue. This requirement has been satisfied in two ways: First, functionally, through the demonstration that AA, on addition to isolated arteries, produces dose-dependent vasodilatation that can be blocked by inhibition or depletion of one or more components of the cytochrome P-450 system, and, second, chemically, based on gas chromatographic/mass spectrometric criteria for the identification of P-450-AA metabolites in vascular tissues.

Pharmacological Methods Used to Study P-450 Vascular Mechanisms

In studies on possible participation of P-450-AA metabolites in vascular mechanisms, experimental designs based on the use of drugs with predictable effects on the P-450 system have proved invaluable. The following experimental design (Figure 3) has greatly accelerated the pace of our studies:

1. Inhibition of cyclooxygenase with indomethacin, in low concentrations (1–3μM), will not diminish the biological actions of AA when it acts via transformation by P-450-dependent oxygenation (63). On the other hand, indomethacin may amplify a P-450-dependent vascular mechanism mediated by AA metabolites, probably, by eliminating a prostaglandin inhibitory modulator of the P-450-AA pathway, possibly PGE_2 (48). The

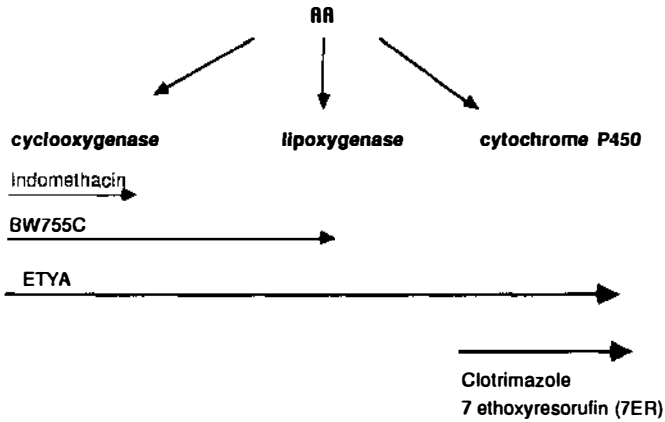

Figure 3 Schematic representing the inhibitory effects of various agents on the enzymes involved in arachidonic (AA) metabolism via cyclooxygenase (inhibited by indomethacin, BW755C, and ETYA), lipoxygenase (inhibited by BW755C and ETYA) and the cytochrome P-450 pathway (inhibited by ETYA). Clotrimazole and 7ER inhibit only the cytochrome P-450 pathway.

alternative explanation that cyclooxygenase inhibition results in diversion of more AA into P-450 pathways, if it operates, appears to be a minor component. Indomethacin may even uncover a latent cytochrome P450-dependent vascular action of AA as it did in the rat kidney (58) and in the rat intestines (64).

2. BW755C (3-amino-1-[m-(trifluoromethyl)-phenyl]-2-pyrazoline), the dual inhibitor of lipoxygenases and cyclooxygenase, was used to eliminate the participation of a lipoxygenase pathway (65). It should not affect P-450-dependent conversion of AA and the attendant biological actions.
3. The final step in this pharmacological sequence was to determine the effects of eicosatetraynoic acid (ETYA) (3) on the action of AA, having demonstrated invulnerability of the biological response to AA to successive attempts at blockade by indomethacin and BW755C. The presumptive P450-AA-mediated biological action should be nullified by ETYA, as in our hands ETYA inhibited the three pathways of AA metabolism in all tissues tested thus far.

Based on the sequential effects of indomethacin, BW755C, and ETYA on the biological response to AA, a cytochrome P-450 pathway can be tentatively identified (Figure 3). The next step is to demonstrate that inhibition of cytochrome P-450 oxygenases can prevent generation of P-450-AA metabolites. Finally, as P-450-AA metabolite formation can be manipulated by pharmacological means (29), it should be possible to amplify or diminish the

biological effects of AA metabolism via P-450 monooxygenases by agents that induce P-450 oxygenase activity or deplete cytochrome P-450 content, respectively.

The above experimental design has been used by Oyekan et al (58) to examine a latent cytochrome P-450-dependent vasodilator response to AA in the rat kidney. The usual vascular response of the rat kidney to AA is constriction mediated by prostaglandin endoperoxides (66). Renal vasoconstriction elicited by AA can be converted to dilatation either by inhibition of cyclooxygenase or blockade of prostaglandin endoperoxide receptors. Elevation of renal perfusion pressure from ca 80 to 180 mmHg by infusion of phenylephrine greatly potentiated the renal vasodilator effect of AA (58). The renal vasodilator action of AA was unaffected by BW755C, whereas ETYA prevented it, suggesting the presence of an oxygenase, neither cyclooxygenase nor lipoxygenase.

INHIBITORS OF P-450-AA METABOLISM Interpretation of the effects of inhibitors of cytochrome P-450 oxygenases on the latent renal vasodilator action of AA, as described above, was complicated by unwanted side effects, mostly avoidable if appropriate doses of the inhibitor were used. The prototypical inhibitor of the P-450 system, β-diethylaminoethyl-diphenylpropylacetate hydrochloride, (SKF525A) (67), although preventing AA-induced renal vasodilatation, also blunted the vasodilator responses to acetylcholine and nitroprusside that were used to define selectivity of inhibitors. However, 7-ethoxyresorufin (7-ER) and clortrimazole, putative inhibitors of cytochrome P-450 monooxygenases (32, 63), possessed the requisite specificity. They inhibited the renal vasodilator response to AA without affecting the dilator actions of either acetylcholine or nitroprusside on the renal vasculature. This study illustrates the need to demonstrate selectivity for a given dose of an agent reported to inhibit the cytochrome P-450 system under a given set of experimental conditions and for the species and tissue/organ under study, as doses vary according to these determinants. The development of specific inhibitors of several P-450 isozymes has been reported and is based on designing analogs of the natural substrates (68).

The endothelial contribution to the renal vasodilator response elicited by AA was judged to be large, as chemical denudation of the endothelium with 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulphonate dihydrate (CHAPS) greatly reduced the renal vasodilator response to AA (and, as expected, to acetylcholine) without affecting that to nitroprusside. As endothelial dependency of the AA-induced renal vasodilatation was shown, it was necessary to exclude EDRF participation in the vasodilator response to AA under these experimental conditions. Methylene blue, which inhibits guanylate cyclase (69), was without affect on AA-induced renal vasodilation, whereas the renal vasodilator effects of acetylcholine and nitroprusside that

act through an EDRF/guanylate cyclase mechanism (70) were notably diminished. These effects of methylene blue eliminated the participation of EDRF in the vasodilator response to AA in the rat kidney.

In a companion study, perturbations of the cytochrome P-450 system of the rat kidney either magnified the renal vasodilator effect of AA (by inducing enzyme activity with either β-NF and 3MC or with glucocorticoid treatment) or dampened it (by depleting renal cytochrome P-450 with $CoCl_2$) (71). These interventions achieved their intended effects; they produced corresponding changes in cytochrome P-450-related enzyme activity in vascular tissues and thereby altered AA metabolism by this pathway.

BIOASSAY OF P-450-AA METABOLITES The study of Oyekan (58) was complemented by the demonstration of the release into the venous effluent of vasorelaxant material from the kidney by AA. The renal effluent cascaded over aortic, celiac, and mesenteric arterial rings that monitored release from the kidney of AA metabolites capable of relaxing blood vessels. Altered release into the renal effluent of P-450-AA metabolites that relaxed blood vessels, as detected by bioassay, corresponded to changes in the renal vasodilator response to AA and thereby established an identity between the vasorelaxant material in the renal venous effluent and renal vasodilator P-450-AA metabolites. This, in turn, has facilitated characterization of the relevant P-450-AA metabolite(s) and should lead to their definitive structural analysis. The first step towards this objective has been realized. Several peaks of P-450-AA products have been identified in extracts of renal venous effluent after separation by reverse phase HPLC. Once again, the utility of superfusion bioassay, as developed by Vane et al (72) for detection and characterization of unknown mediators, is confirmed.

EXTRARENAL VASCULAR CYTOCHROME P-450 MECHANISMS Endothelial-dependent relaxation of rings of the rabbit aorta in response to AA was potentiated after inhibition of cyclooxygenase with indomethacin and increasing basal tension with phenylephrine (73). It was attenuated by agents, nordihydroguariaretic acid and ETYA, that can inhibit the cytochrome P-450 pathway (3). A cytochrome P450-dependent relaxation of the rabbit pulmonary artery elicited by AA was also unmasked, but only after increasing basal tension with phenylephrine, as was the case with the rabbit aorta (74). Under conditions of low basal tension only a vasoconstrictor cyclooxygenase product was formed in response to AA. After elevating tension of the pulmonary artery with phenylephrine, AA produced vasorelaxation having two components: the first, endothelial-dependent, was inhibitable with SKF 525A; the second was endothelial-independent and eliminated by indomethacin. In accordance with a cytochrome P-450 pathway partially determining the en-

dothelial-dependent responses to AA, either amplification or attenuation of AA-induced relaxation of pulmonary artery rings was produced by changing the activity of cytochrome P-450 monooxygenases upward or downward, respectively.

A SPECTRUM OF VASCULAR P-450-DEPENDENT MECHANISMS? A spectrum of cytochrome P-450-dependent mechanisms has been described within the vasculature: muscular arteries such as the femoral have the lowest representation and coronary arteries have among the highest, with the aorta intermediate (75). Indeed, the capacity of AA to elicit a cytochrome P-450 dependent vascular relaxation in these blood vessels correlated with the level of P-450 enzyme activity (measured as AHH activity). In the femoral artery, AA-induced endothelial-dependent relaxation was mediated by a cyclooxygenase product, as indomethacin abolished this effect of AA (76). In contrast, in the coronary artery, indomethacin did not eliminate the vasorelaxorant effect of AA; rather parallel displacement of the AA dose-response curve occurred after cyclooxygenase inhibition, indicating a significant component generated by a non-cyclooxygenase pathway of AA metabolism (75). Based on the effects of inhibitors on the second component of the response to AA in coronary arteries, a P-450-AA metabolite was considered to be the mediator of vasorelaxation.

A spectrum of endothelial-dependency linked to P-450 oxygenase activity was also distinguished among several agonists when tested on isolated coronary arteries (75). Vascular relaxation produced by AA showed the greatest dependency on linkage to P-450 oxygenase. Acetylcholine-induced relaxation of the coronary artery was independent of this mechanism, whereas relaxation produced by moderate doses of bradykinin was intermediate in the degree of dependency on a P-450 mechanism. As the dose of bradykinin increased, a non-AA-related factor, presumably EDRF, became evident as a mediator of the relaxation of coronary arteries to the peptide.

Physiological Aspects of the Vascular P-450 System

FUNCTIONAL HYPEREMIA In terms of potential physiological relevance of the vascular P-450 system, a study by Proctor et al (64) on the intestinal circulation linked a physiological response, hyperemia evoked by nutrient absorption, to mediation by a P-450-AA metabolite. They have provided evidence that a non-cyclooxygenase product mediated the intestinal hyperemia as it was potentiated by inhibition of cyclooxygenase. The application of AA to the mucosa of the rat small intestines mimicked the increased intestinal blood flow caused by nutrient absorption, whereas AA did not register this affect if applied to the serosa. Of the EETs tested for their

capacity to elicit intestinal hyperemia, 5,6-EET was the most potent, surpassing that of adenosine, the reference vasodilator agent.

FETAL BLOOD VESSELS The cytochrome P-450 system in the vasculature can also subserve vasoconstrictor mechanisms, as appears to be the case for fetal vascular shunts—the ductus arteriosus and ductus venosus—on exposure to increased pO_2. Coceani & Olley and their colleagues (77, 78) have made a strong case for involvement of an AA metabolite generated by an intramurally sited cytochrome P-450-dependent oxygenase in the closure of each ductus, whereas patency of these fetal blood vessels appears to be determined by a vasodilator prostaglandin. Carbon monoxide and other inhibitors of the cytochrome P-450 system relaxed each ductus. In contrast to studies on adult blood vessels, removal of the ductal endothelium was without effect on contractile tension of the ductus produced by increased pO_2, suggesting that the relevant cytochrome P-450-dependent oxygenase in fetal vascular shunts is found in the media, perhaps in addition to the intima. Other differences mark fetal and adult vascular tissues in terms of the P-450 system, such as susceptibility to blockade by Type I vs Type II inhibitors that affect cytochrome P-450 by different mechanisms. Binding to the protein moiety of cytochrome P-450 characterizes Type I inhibitors, of which the prototype is SKF 525A, and lipophilic binding to the heme iron characterizes metyrapone, a Type II inhibitor (79, 80). The distinctions between fetal and adult cytochrome P-450 have been interpreted as foreshadowing differences in maturation, functional expression, distribution intramurally, and product formation of "two distinct cytochrome P-450 systems" (78).

MYOGENIC RESPONSE OF BLOOD VESSELS The presence of a cytochrome P-450 system in blood vessels has potentially broad functional implications that should result in understanding vascular mechanisms, heretofore only characterized in a descriptive manner. For example, the contractile response to elevated transmural pressure in vascular beds, the myogenic response first described by Bayliss (81), is a key mechanism in the regulation of the circulation. Evidence based on prevention of increases in arterial wall tension in response to elevated perfusion pressure by inhibitors of P-450-AA synthesis suggests that a P-450-AA product mediates the myogenic response (82). Thus, agents that either prevented AA release by inhibiting phospholipase A_2 or reduced metabolic transformation of AA by the cytochrome P-450 system blunted the development of active tension in dog renal arteries subjected to elevated transmural pressure. Indomethacin and a thromboxane-prostaglandin endoperoxide receptor antagonist were without effect on the myogenic response.

In a related study, the myogenic response has been redefined for cerebral

blood vessels. A transferable contractile factor, with characteristics similar to those of a P-450 product, is released from cat cerebral arteries subjected to increased intramural pressure, and can act on downstream segments (83). The factor was of endothelial origin and diffusible; it depolarized vascular smooth muscle. Moreover, the contractile response had many of the temporal characteristics of an arachidonate-dependent mechanism. It was generated rapidly in response to pressurization, and its release and activity were readily reversible on removal of the stimulus. Formation of the factor may also be promoted by severe hypoxia, which has been reported to release an endothelial-derived contractile substance (84).

VASCULAR OXYGEN SENSORS The studies cited above lead to a reconsideration of vascular oxygen sensors in terms of characteristics shared with the vascular representation of the cytochrome P-450 system: (*a*) The studies on the ductus arteriosus (77, 78) are in accord with a cytochrome P-450 link in the vascular response to changes in pO_2, as is the case with hypoxic pulmonary vasoconstriction (85); (*b*) Endothelial localization of a putative oxygen sensor was apparent from the study of Busse et al (86) who abrogated the response of the rat tail artery to hypoxemia by removing the endothelium; (*c*) A non-cyclooxygenase AA metabolite has been reported to mediate the response of the microcirculation to changes in pO_2 (87).

Pathophysiological Aspects of the Vascular P-450 System

The pathophysiology of the vascular P-450 system is just beginning to be explored. Two highly significant recent studies attempt to relate tissue injury to the participation of the cytochrome P-450 system within the vasculature. The first study was based on an analysis of the dilator response of the microcirculation of the hamster cheek pouch, which resembles that of the skin, to bum injury (21). Based on the susceptibility of the bum-induced vasodilatation to modification by inhibitors of AA metabolism and using an experimental design similar to that described above in the study of Oyekan et al (58), a cytochrome P-450-AA product was considered to be the most likely mediator of the cutaneous vasodilatation produced by thermal injury. As 12(R)-HETE was identified in the skin (88) and was shown to be the principal eicosanoid found in the cutaneous lesions of psoriasis (89), it was tested for its capacity to dilate the cheek pouch. It proved to be a potent dilator of the cheek-pouch vasculature, as was 5,6-EET (21).

The second study addressed the effects of canine coronary artery stenosis on production of AA metabolites within the wall of stenosed arteries. Qualitative and quantitative changes in AA metabolism were produced by coronary artery stenosis. All four EETs and their vicinal diols were increased in stenosed arteries (90). Further, a five-to-tenfold increase in the synthesis of

12- and 15-HETE occurred in response to stenosis; the level of 12-HETE in coronary arteries was tenfold greater than that of 15-HETE. These findings may partially explain the origin of the greatly enhanced 12-HETE levels, in addition to the presumed contribution of activated platelets (34) observed in canine hearts subjected to ischemic injury (91).

HYPOXIC PULMONARY VASOCONSTRICTION The cytochrome P-450 system in the lung has been postulated to mediate hypoxic-induced pulmonary vasoconstriction. Sylvester & McGowan (85) have related diminished binding of O_2 by the pulmonary cytochrome P-450 system, (i.e. increased desaturation of cytochrome P-450) to pulmonary vasoconstriction, and have proposed a chemical mediator of this effect. A priori, constriction of pulmonary blood vessels by hypoxia could result from diminished production of a vasodilator agent, perhaps related to the P-450-AA metabolite implicated in the vasorelaxant effect of isolated pulmonary arteries (see above). However, the inhibitory action of CO and metyrapone on hypoxic vasoconstriction favors a vasoconstrictor P-450-AA metabolite mediating the pulmonary vascular response to hypoxia. Alternatively, the production of a vasoconstrictor P-450-AA metabolite may act in concert with the first mechanism, inhibition of a vasodilator AA product, under hypoxic conditions in the lung. Several P-450-AA metabolites are capable of constricting blood vessels (15). However, variations in their vasoactivity amongst species and vascular beds are prominent.

P-450-AA METABOLITES ACTING AS MEDIATORS OF VASCULAR MECHANISMS

The above survey of vascular mechanisms, possibly subserved by the cytochrome P-450 system, leads to an examination of P-450-AA metabolites with prominent vascular actions that could act as mediators of mechanisms operating within blood vessels. Blood vessels demonstrated a wide range of biosynthetic capacity and diversity with regard to P-450-AA products. Quantitative and qualitative differences depend on the species, the vascular bed, and the presence of injury (90). Moreover, the vascular response to P-450-AA metabolites varies with the vascular bed, the species, and the experimental conditions. For example, 12(R)-HETE is a potent renal vasoconstrictor (92), whereas it was without activity on canine coronary arteries (90), and dilated the microcirculation of the hamster cheek pouch (21). 12(R)-HETE also exhibited vascular actions related to its capacity to inhibit Na^+-K^+-ATPase, such as attenuation of K^+-induced relaxation of vascular rings previously exposed to K^+-free medium (93).

The vascular actions of 20-HETE and 5,6-EET, unlike those of 12-HETE,

were reported to be expressed only after conversion by cyclooxygenase to prostaglandin analogs (18, 41; Figure 2). Oliw (94) recognized that 5,6-EET, which retains the 8, 11, and 14 position (cis) double bonds of its parent compound, AA, was a substrate for cyclooxygenase and could be metabolized to prostaglandin analogs. Alone of the EETs, the 5,6-EET dilated the rat caudal artery (95), an effect that was endothelial-dependent (18). The latter was a function of transformation of the 5,6-EET by endothelial cyclooxgenase as noted, and provides a mechanism for coupling transport effects of unmodified 5,6-EET to changes in local blood flow produced by the metabolism of 5,6-EET via cyclooxygenase in blood vessels. This mechanism may exist in the S_1 segment of the renal proximal tubules where 5,6-EET has been suggested to mediate the natriuretic action of high dose angiotensin II (36). On extrusion into the extracellular space, 5,6-EET can be acted on by vascular cyclooxygenase, resulting in dilatation of the peritubular vascular network. Thus, tubular and vascular function locally within the region or zone may be coordinated by an eicosanoid acting initially as a second messenger within the cell of origin and then undergoing transformation to a product exhibiting vasoactivity within the local environment and perhaps acting systemically.

Ellis et al (96) proposed an alternative mechanism for the expression of 5,6-EET vasoactivity, based on metabolism of the epoxide by the peroxidase activity of prostaglandin synthase, which generates free radicals of oxygen possessing vasodilator properties. In addition to cyclooxygenase, prostaglandin synthase contains a peroxidase that converts the hydroperoxide PGG_2 to PGH_2, the hydroxyl endoperoxide, associated with the formation of oxygen radicals (97). Inhibitors of the cyclooxygenase activity of prostaglandin synthase prevented PGG_2 formation and, secondarily, inhibited oxygen radical-generation (96). In support of free radicals acting as the vasoactive species in the cerebral microcirculation, Ellis et al (96) demonstrated that, similar to indomethacin, the free radical scavengers, superoxide dismutase plus catalase, prevented dilatation of the rabbit and cat pial arterioles in response to 5,6-EET. In this circulatory bed, other EETs had negligible effects. Further, the cerebral vasodilator response to AA may also be mediated by the same mechanism, generation of oxygen radicals, evoked by 5,6-EET, as it was nullified by either indomethacin or free radical scavengers (96). This study assumes physiological significance as 5,6-EET constitutes a significant portion of products synthesized from AA by brain slices.

These studies on 5,6-EET (18) and 20-HETE transformation (98) by cyclooxygenase (see below) mandate reinterpretation of the effects of cyclooxygenase inhibitors on eicosanoid mechanisms. Previously, aspirinlike compounds, if they inhibited a biological action of either AA or of an agent thought to release AA, such as vasoactive peptides and amines, were consid-

ered to act by eliminating the prostanoid component from the response to the agonist. This interpretation is no longer tenable if a cell or tissue can be shown to have significant capacity to generate P-450-AA products that can be metabolized by cyclooxygenase, the actions of which AA products are dependent on the cyclooxygenase step.

Recent studies require the cyclooxygenase-dependency of the vasoactivity of 5,6-EET to be qualified in terms of specific vascular beds and species. The EETs, including 5,6-EET, have been reported to relax precontracted canine coronary arteries despite inhibition of cyclooxygenase (90). Moreover, the four EETs were equipotent in their coronary vasorelaxing ability; these findings stand in contrast to those in the caudal artery (95), the hamster cheek pouch (21), the ductus arteriosus (77), and the cerebral (96) and intestinal microcirculations (64). In these vasculatures, 5,6-EET was the most potent vasodilator of the EETs; the other EETs were usually inactive.

Another exception to the superior potency of 5,6-EET when compared to the other EETs is in their ability to inhibit platelet aggregation. Fitzpatrick et al (15, 99) have studied the platelet antiaggregatory properties of the EETs; 14,15-EET was the most potent. These investigators demonstrated that 14,15-EET inhibited purified cyclooxygenase; however, the latter property could be dissociated from the ability to inhibit platelet aggregation. Thus, concentrations of 14,15-EET that inhibited platelet aggregation were without affect on platelet thromboxane formation. These observations on platelets were extended to neutrophils as aggregation of neutrophils that lack cyclooxygenase is not affected by aspirinlike drugs (15). As neutrophil aggregation was inhibited by 14,15-EET, it was concluded that the antiaggregatory mechanism was independent of cyclooxygenase and may be linked to a signal transduction pathway. It should be noted that the platelet studies were conducted on washed human platelets. When platelet-rich plasma was used, the concentration of the EET required to inhibit aggregation was greatly increased, suggesting catabolism or protein binding of EETs by plasma (90).

An additional caveat concerning the vasoactive properties of epoxides and linkage to cyclooxygenase relates to species differences. In the rat, 5,6-EET infused into the renal artery produced dose-dependent reductions in GFR and renal blood flow (27) whereas in the rabbit a renal vasodilator response to 5,6-EET occurred (100). Further, in the rat kidney, inhibition of cyclooxygenase reversed the hemodynamic response to 5,6-EET; GFR and renal blood flow increased in response to the epoxide (27). In contrast, indomethacin eliminated the vasoactivity of 5,6-EET in the rabbit kidney (100). A possible explanation for the dissimilar renovascular responses to 5,6-EET in the rat and rabbit is based on the determinants of the renovascular responses of the rat and rabbit to AA, which also elicited dissimilar vascular effects in these species (101). In the rat, AA caused renal vasoconstriction mediated by

prostaglandin endoperoxides (66), whereas the characteristic response of the rabbit kidney to AA, vasodilation, was mediated by vasodilator prostanoids requiring transformation of the endoperoxides (101). A ready interpretation of these differences is that in the rabbit kidney, but not in the rat kidney, facile transformation of the endoperoxide PGH_2 to vasodilator protanoids is the rule. By analogy, in the rat kidney, the initial formation of the vasoconstrictor endoperoxide analog from 5,6-EET proceeds no further, whereas in the rabbit kidney the endoperoxide analog undergoes extensive additional metabolism to vasodilator metabolites, e.g. PGE and/or PGI analogs of 5,6-EET.

P-450-AA METABOLITES AND TUBULAR TRANSPORT MECHANISMS

In the mTALH

A similar metabolic sequence acting via cyclooxygenase confers vasoactivity on 20-HETE, which was initially described as a vasoconstrictor P-450-AA metabolite. Escalante et al (41) have obtained evidence that the vasoconstrictor effect of 20-HETE depends on its transformation to a prostaglandin endoperoxide analog. This capability has taken on added significance in view of recent findings that identify 20-HETE as a major product of P-450-AA metabolism in mTALH where it, or one of its metabolites, modifies transport function in this tubular segment (48). We had reported in 1985 that mTALH cells isolated from rabbit generated AA metabolites via a cytochrome P-450 dependent monooxygenase (3) and that these AA products segregated into two peaks on reverse phase HPLC, one containing principally vasodilator material, and the second containing material capable of inhibiting Na^+-K^+-ATPase (45; Figure 4). Two principal AA products, 20-HETE and 20-COOH-AA, have been identified in mTALH (48). The former accounts for the vasoactivity of the less polar peak and may serve as the precursor for 20-COOH-AA (as each AA metabolite can affect transport function in mTALH).

To establish a physiological role for these AA metabolites in mTALH, Escalante et al examined the linkage between changes in transport and changes in AA metabolism in mTALH (48). Perturbations of the renal P-450 system, first shown to affect AA product formation by rabbit renal cortical and medullary microsomes, have been applied successfully to modifying the flux of P-450-AA metabolites by intact mTALH cells (29, 48) in a predictable manner. Changes in P-450-AA metabolism can thus be correlated with altered transport function of mTALH cells. The effects of P-450-AA metabolism on transport was determined by measuring ^{86}Rb uptake in mTALH cells while manipulating the flux of AA through the P-450 system (Figure 5). As ^{86}Rb

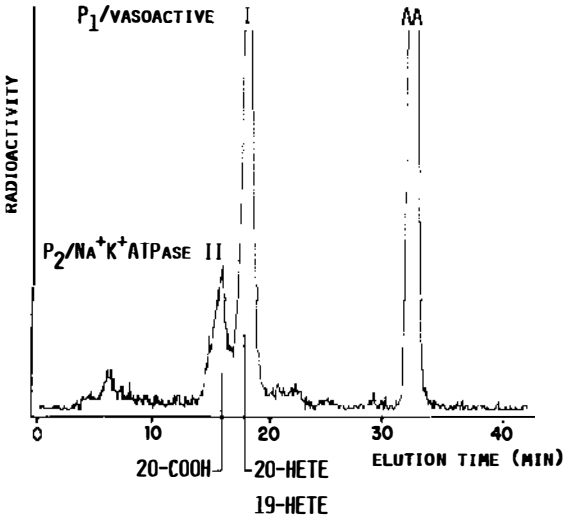

Figure 4 Separation by reverse-phase HPLC of ^{14}C-labeled oxygenated metabolites of arachidonic acid (AA). Medullary thick ascending loop of Henle (mTALH) cells (3×10^6/ml) were incubated with ^{14}C-AA (0.4μCi) for 30 minutes. Cells were removed by centrifugation and the media extracted. AA metabolites were separated on a μ Bondapak C_{18} column, using a linear solvent gradient ranging from water: acetonitrile (1 : 1, containing 0.1% acetic acid) to acetronitrile (containing 0.1% acetic acid); the rate of change was 1.25% per/minute at a flow rate of 1ml/minute. Radioactivity was monitored continuously using a radioactive flow detector.

Structural analysis by gas chromatography-mass spectrometry of the principal product in each peak disclosed 20-hydroxyeicosatetraenoic acid (20-HETE) in peak I (P_1) and 20-carboxy-AA (20-COOH) in peak II (P_2), accounting for vasoactivity and the capacity to inhibit Na^+, K^+ATPase, respectively. Lesser amounts of 19-HETE were identified in peak I.

movement reflects that of K^+, and as K^+ is cotransported with Na^+ and Cl^- in mTALH, ^{86}Rb uptake is a reliable estimate of mTALH transport function. Addition of AA produced a concentration-dependent inhibition of ^{86}Rb uptake, an effect that was prevented by selective blockade of AA metabolism by P-450 pathways. The blockade could be circumvented by the addition of the relevant AA metabolites that were identified as principal products of the cytochrome P-450-dependent pathway of AA metabolism in mTALH. As increased cAMP inhibits Na^+-K^+-ATPase (102) and stimulates P-450-AA metabolism in mTALH cells (45), we concluded that a P-450-AA metabolite is the vital link in these events. Thus, an endogenous modulator of Na^+,K^+-ATPase is generated by cytochrome P-450 metabolism of AA in mTALH, the production of which is increased by cAMP. We propose that the AA-derived modulator of Na^+-K^+-ATPase, generated by a P-450 isozyme, is either 20-HETE or 20-COOH-AA.

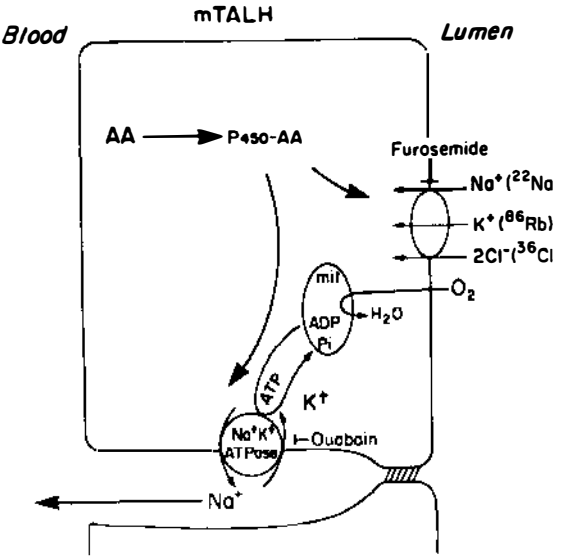

Figure 5 A model for the effect of P-450-AA metabolites on Na^+ transport in the thick ascending limb of Henle's loop in the rabbit kidney medulla. Na^+ transport occurs across the luminal membrane via $Na^+K^+Cl^-$ cotransporter (furosemide inhibitable) and then is extruded from the cell via the Na^+K^+ATPase (oubain inhibitable) in the basal-lateral membrane. O_2 is required for the ATP hydrolysis for Na^+K^+ATPase activation. AA metabolism is via a cytochrome P-450 isozyme that generates metabolites which inhibit Na^+/transport either by affecting Na^+/K^+ATPase or the $Na^+K^+Cl^-$ cotransporter, or both.

In the Proximal Tubules

In addition to the mTALH, the proximal convoluted tubules, particularly the S_1 segment, contain a highly active cytochrome P-450 monooxygenase(s) (36). Douglas et al (103) have identified 5,6-EET as the main product of AA metabolism in this nephron segment, which is also the site of a principal action of angiotensin II (AII) on sodium transport. All increased formation of 5,6-EET, probably by stimulating phospholipase A_2 and thereby promoting the delivery of free AA to the oxygenase. They have obtained evidence that this mechanism represents the means for expression of the natriuretic action of high dose (ηM-μM) AII. Thus, high dose AII, as well as authentic 5,6-EET, inhibited unidirectional ^{22}Na flux (J_{Na}). Ketoconazole, an inhibitor of epoxygenase (63), prevented the action of AII on sodium movement and potentiated that of authentic 5,6-EET, results in accord with the interpretation that 5,6-EET acts as the second messenger of the AII effect on sodium transport. The linkage of 5,6-EET to inhibition of sodium transport was examined in terms of inhibition of Na^+,-K^+-ATPase and rejected, as 5,6-EET was a weak inhibitor of the enzyme. However, 5,6-EET increased cytosolic calcium

dose-dependently, having a threshold concentration of 10^{-8}M. The epoxide promoted calcium influx through voltage-sensitive channels; this in turn was thought to facilitate calcium-calmodulin related inhibition of the Na^+-H^+ exchanger on the luminal membrane of the proximal tubules. The effects of EETs on calcium mobilization may explain many of the actions of these P-450-AA metabolites.

P-450-AA METABOLISM IN HYPERTENSION

As the mTALH generates P-450-AA metabolites capable of affecting Na^+-K^+-ATPase activity, these eicosanoids may participate in rapid adjustments of sodium reabsorption or potassium secretion by the renal tubules. An endogenous modulator of Na^+-K^+-ATPase activity has been long sought, having been postulated to account for rapid adjustments in sodium reabsorption and potassium excretion by the nephron (104). Likewise, the operation of a factor in the mTALH segment in hypertension has been advanced to account for decreased activity of Na^+-K^+-ATPase in the outer medulla (105). The latter is expressed functionally in hypertension by disturbances in the absorption of sodium chloride as, for example, accelerated excretion of a sodium chloride load in hypertensive man (106). It should be recalled that hypertension has been proposed to be a secondary event, the primary or initiating event being increased sodium chloride absorption that was offset by elevated renal perfusion pressure associated with systemic hypertension. This was first proposed by Baldwin et al (107) and received support from the system analysis developed by Guyton & Coleman (108), who concluded that renal excretory impairment was central to the development of hypertension. The elevation of blood pressure was, therefore, compensatory to facilitate sodium chloride excretion. Sacerdoti et al (109) have obtained evidence that this mechanism operates in the SHR, as normalization of the relationship between sodium excretion and renal perfusion pressure was produced by reducing the generation of pro-hypertensive P-450-AA metabolites.

The cytochrome P-450 pathway of AA metabolism in the mTALH is highly selective in responding to circulatory hormones (45) and demonstrates large changes in product formation when challenged by either renal ischemia or changes in salt intake (50, 51). The interaction of hormones with the cytochrome P-450 system in the nephron has broad implications regarding the regulation of the volume of body fluids and the control of blood pressure. For example, the cytochrome P-450-dependent pathway of AA metabolism in mTALH may act as a common mechanism mediating changes in sodium and chloride excretion in response to hormones and to changes in renal perfusion pressure, as well as to natriuretic substances including circulating inhibitors of Na^+-K^+-ATPase activity, which may be elevated in hypertension (110).

P-450-AA Metabolism in the SHR

Experimental interventions, as noted, are available to modify the flux of AA through the renal cytochrome P-450 system, making it possible to address the contribution of P-450-AA products to renal functional disturbances in hypertension. A renal lesion has been proposed as the cause of high blood pressure not only in essential hypertension in humans but also in the spontaneously hypertensive rat (SHR), the most studied animal model of human hypertension (111). In the SHR, salt and water retention and depression of GFR and renal blood flow coincide with the rapid developmental phase of hypertension, between 5 to 13 weeks of age, and disappear in the mature SHR (112, 113). Eicosanoid-related renal functional abnormalities, particularly as they relate to products of the cyclooxygenase pathway, have been examined in terms of their participation in the pathogenesis of hypertension in the SHR (111). However, prostaglandin-dependent mechanisms have been found wanting as primary factors in the pathogenesis of hypertension in the SHR; rather they appear to be secondary to the elevation of blood pressure. Because of the prohypertensive properties of several P450-AA products, including vasoconstriction (41) and stimulation of Na^+-K^+-ATPase activity (114), we examined the SHR for abnormalities of P-450-AA metabolism. Increased renal production of ω-/ω-1 HETEs occurred in the SHR during the developmental phase of hypertension (115). As renal cytochrome P-450-dependent AA metabolism can be depressed selectively with $SnCl_2$ (116), we used $SnCl_2$ to determine the effects of decreased formation of renal P-450-AA metabolites on the elevated blood pressure of the SHR. $SnCl_2$, as $CoCl_2$, induced renal heme oxygenase, but, unlike $CoCl_2$, $SnCl_2$ did not affect heme oxygenase of extrarenal tissues (22). As heme oxygenase is the rate-controlling enzyme in heme catabolism, it reduces the availability of heme for hemeoprotein such as cytochrome P-450 monooxygenases and results in diminished cytochrome P-450 content, and, secondarily, decreased metabolism of AA through cytochrome P-450 pathways (22).

Acute treatment with $SnCl_2$ was antihypertensive in 6-week-old SHRs, requiring two days to normalize blood pressure (109). Chronic treatment prevented the development of hypertension in the young SHR (7). In either instance reduction of blood pressure was obtained only if $SnCl_2$ was given before the established phase of hypertension. $SnCl_2$ was without effect on blood pressure in either young or adult normotensive WKY rats or, as noted, in adult SHRs. $SnCl_2$ resulted in decreased formation of ω-(20-HETE) and ω-1-(19-HETE) hydroxylation products by the renal cytochrome P-450 pathway in the SHR whereas formation of EETs did not appear to be affected (109). Reduced formation of P-450-AA products in 6-week-old SHRs occurred concomitantly with a fall in blood pressure and increased excretion of sodium chloride (22). Natriuresis-chloruresis, therefore, occurred despite a fall in blood pressure, changes compatible with normalization of the relation-

ship between blood pressure and sodium chloride excretion. That is, the blood pressure-natriuresis curve was shifted to the left (108) in response to $SnCl_2$-induced recovery from hypertension. The effect of $SnCl_2$ was consistent with the proposal that hypertension acts as a compensatory mechanism to restore sodium chloride hemeostasis in the face of enhanced activity of factors promoting salt reabsorption and arising from a cytochrome P-450 isozyme.

As renal cytochrome P-450-dependent monooxygenases can also generate mineralocorticoids with the capacity to elevate blood pressure, it is important to recognize that products of the renal cytochrome P-450 system, other than AA metabolites, may participate in the elevation of blood pressure in the SHR. Melby and colleagues (117) have reported that a prohypertensive mineralocorticoid, 19-nor-deoxycorticosterone, can be generated by the cytochrome P-450 system in increased amounts in the young SHR.

SUMMARY

The cytochrome P-450 pathway of AA metabolism is widely distributed and gives rise to a diversity of products affecting basic biological mechanisms such as vascular reactivity and transport function in critical nephron segments. P-450-AA metabolites may participate in receptor-mediated signal transduction and may act as second messengers. The synthesis of P-450-AA products can be altered by pharmacologic probes and is affected by pathophysiological conditions. That this review has centered on the circulation and renal function should not be interpreted as minimizing the importance of P-450-AA metabolites in other organs and systems for, most assuredly, they will be shown to constitute an essential component of organ function in sites other than those addressed here.

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Literature Cited

1. Capdevila, J. H., Falck, J. R. 1989. Cytochrome P-450 and the bioactivation of arachidonic acid. *Blood Vessels* 26:54–57
2. Schwartzman, M., Carroll, M. A., Ibraham, N. G., Ferreri, N. R., Songu-Mize, E., et al. 1985. Renal arachidonic acid metabolism (The third pathway). *Hypertension* (Suppl I) 7:1-136-44
3. Ferreri, N. R., Schwartzman, M., Ibraham, N. G., Chander, P. N., McGiff, J. C. 1984. Arachidonic acid metabolism in a cell suspension isolated from rabbit renal outer medulla. *J. Pharmacol. Exp. Ther.* 231:441–48
4. Schwartzman, M. L., Abraham, N. G., Masferrer, J. L., Dunn, M. W., McGiff, J. C. 1985. Cytochrome P450 dependent

metabolism of arachidonic acid in bovine corneal epithelium. *Biochem. Biophys. Res. Commun.* 132:343–51
5. Masferrer, J. L., Schwartzman, M. L. 1989. Novel Cytochrome P450-dependent arachidonic acid metabolites and their ocular effects. In *The Ocular Effects of Prostaglandins and Other Eicosanoids*, ed. L. T. Bito, J. Stjernschantz, pp. 85–93. New York: Liss
6. Abraham, N. G., Pinto, A., Mullane, K. M., Levere, R. D., Spokas, E. G. 1985. Presence of cytochrome P-450-dependent monooxygenase in intimal cells of the hog aorta. *Hypertension* 7:899–904
7. McGiff, J. C. 1990. Regulation of blood pressure by cytochrome P450 arachidonate metabolites. *Hypertension*. In press
8. Williams, D. E., Hale, S. E., Okita, R. T., Masters, B. S. S. 1984. A prostaglandin ω-hydroxylase cytochrome P-450 ($P\text{-}450_{PG\text{-}\omega}$) purified from lungs of pregnant rabbits. *J. Biol. Chem.* 259: 14600–8
9. Haurand, M., Ullrich, V. 1985. Isolation and characterization of thromboxane synthase from human platelets as a cytochrome P-450 enzyme. *J. Biol. Chem.* 260:15059–67
10. DeWitt, D. L., Smith, W. L., 1983. Purification of prostacyclin synthase from bovine aorta by immunoaffinity chromatography. *J. Biol. Chem.* 258: 3285–93
11. Capdevila, J., Chacos, N., Werringloer, J., Prough, R. A., Estabrook, R. W. 1981. Liver microsomal cytochrome P-450 and the oxidative metabolism of arachidonic acid. *Proc. Natl. Sci. USA* 78:5362–66
12. Capdevila, J., Pramanik, B., Napoli, J. L., Manna, S., Falck, J. R. 1984. Arachidonic acid epoxidation epoxyeicosatrienoic acids are endogenous constituents of rat liver. *Arch. Biochem. Biophys.* 231:511–17
13. Oliw, E. H., Guengerich, F. P., Oates, J. A. 1982. Oxygenation of arachidonic acid by hepatic monooxygenases. *J. Biol. Chem.* 257:3771–81
14. Morrison, A. R., Pascoe, N. 1981. Metabolism of arachidonic acid through NADPH-dependent oxygenase of renal cortex. *Proc. Natl. Acad. Sci. USA* 78:7375–78
15. Proctor, K. G., Capdevila, J. H., Falck, J. R., Fitzpatrick, F. A., Mullane, K. M., et al. 1989. Cardiovascular and renal actions of cytochrome P-450 metabolites of arachidonic acid. *Blood Vessels* 26:53–64
16. Fitzpatrick, F. A., Murphy, R. C., 1989. Cytochrome P-450 metabolism of arachidonic acid: Formation and biological actions of "epoxygenase"-derived eicosanoids. *Pharmacol. Rev.* 40:229–41
17. Capdevila, J. H., Karara, A., Waxman, D. J., Martin, M. V., Falck, J. R., et al. 1990. Cytochrome P-450 enzyme-specific control of the regio and enantiofacial selectivity of the microsomal arachidonic acid epoxygenase. *J. Biol. Chem.* In press
18. Carroll, M. A., Garcia, M. P., Falck, J. R., McGiff, J. C. 1990. 5,6-epoxyeicosatrienoic acid, a novel arachidonate metabolite: mechanism of vasoactivity in the rat. *Circ. Res.* 67:1082–88
19. Schwartzman, M. L., Balazy, M., Masferrer, J., Abraham, N. G., McGiff, J. C., et al. 1987. 12(R)-hydroxyeicosatetraenoic acid: A cytochrome P450-dependent arachidonate metabolite that inhibits Na^+,K^+-ATPase in the cornea. *Proc. Natl. Acad. Sci. USA* 84:8125–29
20. Quilley, C. P., McGiff, J. C. 1990. Isomers of 12-hydroxy-5,8,10,14-eicosatetraenoic acid reduce renin activity and increase water and electrolyte excretion. *J. Pharmacol. Exp. Ther.* 254: 774–80
21. Proctor, K. G., Shatkin Jr., S., Kaminski, P. M., Falck, J. R., Capdevila, J. H. 1988. Modulation of arteriolar blood flow by inhibitors of arachidonic acid oxidation after thermal injury: possible role for a novel class of vasodilator metabolites. *Circulation* 77:1185–96
22. Sacerdoti, D., Escalante, B., Abraham, N. G., McGiff, J.C., Levere, R. D., et al. 1989. Treatment with tin prevents the development of hypertension in spontaneously hypertensive rats. *Science* 243:388–90
23. Capdevila, J. H., Kishore, V., Dishman, E., Blair, I. A. 1987. A novel pool of rat liver inositol and ethanolamine phospholipids contains epoxyeicosatrienoic acids (EETs). *Biochem. Biophys. Res. Commun.* 146:638–44
24. Quilley, J., McGiff, J. C. 1988. Renal arachidonic acid metabolism. In *Eicosanoids in the Cardiovascular and Renal Systems*, ed. P. V. Halushka, D. E. Mais, pp. 16–33. Lancaster, Boston, The Hague/Dordrecht: MTP Press
25. Piomelli, D., Volterra, A., Dale, N., Siegelbaum, S. A., Kandel, E. R., et al. 1987. Lipoxygenase metabolites of arachidonic acid as second messengers for presynaptic inhibition of *Aplysia* sensory cells. *Nature* 328:38–43
26. Karara, A., Dishman, E., Blair., I.,

Falck, J. R., Capdevila, J. H. 1989. Endogenous epoxyeicosatrienoic acids. *J. Biol. Chem.* 264:19822–27
27. Takahashi, K., Capdevila, J., Karara, A., Falck, J. R., Jacobson, H. R., et al. 1990. Cytochrome P-450 arachidonate metabolites in rat kidney: characterization and hemodynamic responses. *Am. J. Physiol.* 258:F781–89
28. Falck, J. R., Lumin, S., Blair, I., Dishman, E., Martin, M. V., et al. 1990. Cytochrome P-450 dependent oxidation of arachidonic acid to 16-,17-, and 18-hydroxyeicosatetraenoic acids. *J. Biol. Chem.* 265:10244–49
29. Schwartzman, M. L., Abraham, N. G., Carroll, M. A., Levere, R. D., McGiff, J. C. 1986. Regulation of arachidonic acid metabolism by cytochrome P-450 in rabbit kidney. *Biochem. J.* 238:283–90
30. Schwartzman, M. L., Martasek, P., Rios, A., Levere, R. D., Solangi, K., et al. 1990. Cytochrome P450-dependent arachidonic acid metabolism in human kidney. *Kidney Int.* 37:94–99
31. Oliw, E. H. 1990. A novel arachidonic acid 18(R)-hydroxylase and eicosapentaenoic acid W3-epoxygenase of seminal vesicles. *7th Int. Conf. Prostaglandins and Related Compounds*. Florence, Italy
32. Schwartzman, M. L., Davis, K. L., McGiff, J. C., Levere, R. D., Abraham, N. G. 1988. Purification and characterization of cytochrome P-450-dependent arachidonic acid epoxygenase from human liver. *J. Biol. Chem.* 263:2536–42
33. Falck, J. R., Manna, S., Jacobson, H., Estabrook, R., Chacos, N. et al. 1984. Absolute configuration of epoxyeicosatrienoic acids (EETs) formed during catalytic oxygenation of arachidonic acid by purified rat liver microsomal cytochrome P-450. *J. Am. Chem. Soc.* 106:3334–36
34. Brash, A. R. 1989. 12- and 15-Lipoxygenase products of arachidonic acid at the platelet/vascular interface. In *Platelets and Vascular Occlusion*, ed. C. Patons, G. A., Fitzgerald. New York: Raven
35. Smith, W. L., Bell, T. G. 1978. Immunohistochemical localization of the prostaglandin-forming cyclooxygenase in renal cortex. *Am. J. Physiol.* 235:F451–57
36. Romero, M. F., Douglas, J. G., Hopfer, U. 1990. A P450 epoxygenase metabolite of arachidonic acid mediates angiotensin effects on Na^+ absorption in proximal tubular epithelium. *7th Int. Conf. Prostaglandins and Related Compounds*, Florence, Italy.
37. Hirt, D. L., Capdevila, J., Falck, J. R., Breyer, M. D., Jacobson, H. R. 1989. Cytochrome P450-metabolites of arachidonic acid are potent inhibitors of vasopressin action on rabbit cortical collecting duct. *J. Clin. Invest.* 84:1805–12
38. Hamberg, M., Samuelsson, B., 1974. Prostaglandin endoperoxides. Novel transformations of arachidonic acid in human platelets. *Proc. Natl. Acad. Sci. USA* 71:3400–4
39. Marcus, A. J., Weksler, B. B., Jaffe, E. A., Broekman, M. J. 1980. Synthesis of prostacyclin from platelet-derived endoperoxides by cultured human endothelial cells. *J. Clin. Invest.* 66:979–86
40. Marcus, A. J., Safier, L. B., Ullman, H. L., Broekman, M. J., Islam, N., et al. 1984. 12S, 20-dihydroxyeicosatetraenoic acid: a new icosanoid synthesized by neutrophils from 12S-hydroxyeicosatetraenoic acid produced by thrombin- or collagen-stimulated platelets. *Proc. Natl. Acad. Sci. USA* 81(3):903–7
41. Escalante, B., Sessa, W. C., Falck, J. R., Yadagiri, P., Schwartzman, M. L. 1989. Vasoactivity of 20-hydroxyeicosatetraenoic acid is dependent on metabolism by cyclooxygenase. *J. Pharmacol. Exp. Ther.* 248:229–32
42. Tusell, J. M., Gelpi, E. 1980. Prostaglandins E and F, and 19-hydroxylated E and F (series I and II) in semen of fertile men. *J. Chromatogr.* 181:295–310
43. Wong, P. Y.-K., Malik, K. U., Taylor, B. M., Schneider, W. P., McGiff, J. C., et al. 1985. Epoxidation of prostacyclin in the rabbit kidney. *J. Biol. Chem.* 260:9150–53
44. Oliw, E. 1984. Metabolism of 5,6-oxidoeicosatrienoic acid by ram seminal vesicles: formation of two stereoisomers of prostaglandin I_1. *J. Biol. Chem.* 259:2716–21
45. Schwartzman, M., Ferreri, N. R., Carroll, M. A., Songu-Mize, E., McGiff, J. C. 1985. Renal cytochrome P450-related arachidonate metabolite inhibits $(Na^+ + K^+)$ATPase. *Nature* 314:620–22
46. Jim, K., Hassid, A., Sun, F., Dunn, M. J. 1982. Lipoxygenase activity in rat kidney glomeruli, glomerular epithelial cells, and cortical tubules. *J. Biol. Chem.* 257:10294–99
47. Pinto, A., Abraham, N. G., Mullane, K. M. 1986. Cytochrome P450-dependent monooxygenase activity and endothelial-dependent relaxation in-

duced by arachidonic acid. *J. Pharmacol. Exp. Ther.* 236:445–51
48. Escalante, B., Erlij, D., Falck, J. R., McGiff, J. C. 1990. Cytochrome P450-arachidonate metabolites affect transport in medullary thick ascending limb of the rabbit kidney. *Science*. In press
49. Carroll, M. A., Schwartzman, M., Sacerdoti, D., McGiff, J. C. 1988. Novel renal arachidonate metabolites. *Am. J. Med. Sci.* 295:268–74
50. Carroll, M. A., Schwartzman, M., Baba, M., Miller, M. J. S., McGiff, J. C. 1988. Renal cytochrome P-450-related arachidonate metabolism in rabbit aortic coarctation. *Am. J. Physiol.* 255:F151–57
51. Carroll, M. A., Louzan, M., McGiff, J. C. 1990. K^+ alters cytochrome P-450-dependent arachidonate metabolism by rabbit renomedullary cells. *Am. J. Physiol.* 258:F1084–89
52. Doucet, A., Barlet-Bas, C., Siaume-Perez, S., Khadouri, C., Marsy, S. 1990. Gluco- and mineralocorticoids control adenylate cyclase in specific nephron segments. *Am. J. Physiol.* 258:F812–20
53. Lapuerta, L., Chacos, N., Falck, J. R., Jacobson, H., Capdevila, J. H. 1988. Renal microsomal cytochrome P-450 and the oxidative metabolism of arachidonic acid. *Am. J. Med. Sci.* 295:275–79
54. Kagawa, N., Waterman, M. R. 1990. cAMP-dependent transcription of the human CYP21B($P\text{-}450_{c21}$) gene requires a *Cis*-regulatory element distinct from the consensus cAMP-regulatory element. *J. Biol. Chem.* 265:11299–305
55. Sessa, W. C., Lin, L., Nasjletti, A. 1990. Reciprocal effects of dexamethasone on vasodilatory responses to arachidonic acid and prostanoids in the isolated perfused rabbit kidney. *Hypertension* 15 (Suppl I):I-93–I-96
56. Sessa, W. C., Nasjletti, A. 1990. Dexamethasone selectively attenuates prostanoid-induced vasoconstrictor responses in vitro *Circ. Res.* 66:383–88
57. Endou, H. 1983. Cytochrome P-450 monooxygenase system in the rabbit kidney: Its intranephron localization and its induction. *Jpn. J. Pharmacol.* 33:423–33
58. Oyekan, A. O., McGiff, J. C., Quilley, J. 1991. Cytochrome P450-dependent vasodilator responses to arachidonic acid in the isolated, perfused kidney of the rat. *Circ. Res.* In press
59. Dees, J. H., Masters, B. S. S., Muller-Eberhard U., Johnson, E. F. 1982. Effect of 2,3,7,8-tetrachloro-dibenzo-p-dioxin and phenobarbital on the occurrence and distribution of four cytochrome P-450 isozymes in rabbit kidney, lung, and liver. *Cancer Res.* 42:1423–32
60. Rosolowsky, M., Falck, J. R., Campbell, W. B. 1990. Synthesis and biological activity of epoxyeicosatrienoic acids (EETs) by cultured bovine coronary artery endothelial cells. *7th Int. Conf. Prostaglandins and Related Compounds*. Florence, Italy
61. Moore, S. A., Spector, A. A., Hart, M. N. 1988. Eicosanoid metabolism in cerebromicrovascular endothelium. *Am. J. Physiol.* 254:C37–C44
62. Drugge, E. D., Carroll, M. A., McGiff, J. C. 1989. Cells in culture from rabbit medullary thick ascending limb of Henle's loop. *Am. J. Physiol.* 256: C1070–81
63. Capdevila, J., Gil, L., Orellana, M., Marnett, L. J., Mason, J. I., et al. 1988. Inhibitors of cytochrome P-450-dependent arachidonic acid metabolism. *Arch. Biochem. Biophys.* 261:257–63
64. Proctor, K. G., Falck, J. R., Capdevila, J. 1987. Intestinal vasodilation by epoxyeicosatrienoic acids: Arachidonic acid metabolites produced by a cytochrome P450 monooxygenase. *Circ. Res.* 60:50–59
65. Higgs, G. A., Vane, J. R. 1983. Inhibition of cyclo-oxygenase and lipoxygenase. *Br. Med. Bull.* 39:265–70
66. Quilley, J., McGiff, J. C., Nasjletti, A. 1989. Role of endoperoxides in arachidonic acid-induced vasoconstriction in the isolated perfused kidney of the rat. *Br. J. Pharmacol.* 96:111–16
67. Mannering, G. J. 1971. Microsomal enzyme systems which catalyze drug metabolism. In *Fundamentals of Drug Metabolism and Disposition*, ed. B. LaDu, H., Mandel, E. Woy, pp. 206–52. Baltimore: Williams & Wilkins
68. Muerhoff, A. S., Williams, D. E., Reich, N. O., CaJacob, C. A., Ortiz de Montellano, P. R., et al. 1989. Prostaglandin and fatty acid ω- and (ω-1)-oxidation in rabbit lung. *J. Biol. Chem.* 264:749–56
69. Arnold, W. P., Mittal, C. K., Katsuki, S., Murad, F. 1977. Nitric oxide activates guanylate cyclase and increases guanosine 3' : 5'-cyclic monophosphate levels in various tissue preparations. *Proc. Natl. Acad. Sci. USA* 74:3203–7
70. Ignarro, L. J., Byrns, R. E., Buga, G. M., Wood, K. S. 1987. Endothelium-derived relaxing factor from pulmonary

artery and vein possesses pharmacologic and chemical properties identical to those of nitric oxide radical. *Circ. Res.* 61:866–79

71. Oyekan, A. O., McGiff, J. C., Quilley, J. 1990. Vasodilation mediated by cytochrome P450 derived eicosanoids in the rat kidney. *Counc. High Blood Pressure Res.—44th Ann. Fall Conf. Sci. Sess.*
72. Bunting, S., Gryglewski, R., Moncada, S., Vane, J. R. 1976. Arterial walls generate from prostaglandin endoperoxides a substance (prostaglandin X) which relaxes strips of mesenteric and coeliac arteries and inhibits platelet aggregation. *Prostaglandins* 12:897–913
73. Singer, H. A., Peach, M. J. 1983. Endothelium-dependent relaxation of rabbit aorta. I. Relaxation stimulated by arachidonic acid. *J. Pharmacol. Exp. Ther.* 226:790–95
74. Mullane, K., 1989. Endothelium, vascular tone, and novel metabolites of AA. *Blood Vessels* 26:59–60
75. Pinto, A., Abraham, N. G., Mullane, K. M. 1987. Arachidonic acid-induced endothelial-dependent relaxations of canine coronary arteries: Contribution of a cytochrome P-450-dependent pathway. *J. Pharmacol. Exp. ther.* 240:856–63
76. DeMey, J. G., Claeys, M., Vanhoutte, P. M. 1982. Endothelium-dependent inhibitory effects of acetylcholine, adenosine triphosphate, thrombin and arachidonic acid in the canine femoral artery. *J. Pharmacol. Exp. Ther.* 222: 166–73
77. Coceani, F., Hamilton, N. C., Labuc, J., Olley, P. M. 1984. Cytochrome P450-linked monooxygenase: involvement in the lamb ductus arteriosus. *Am. J. Physiol.* 246:H640–43
78. Adeagbo, A. S. O., Breen, C. A., Cutz, E., Lees, J. G., Olley, P. M., et al. 1990. Lamb ductus venosus: Evidence of a cytochrome P-450 mechanism in its contractile tension. *J. Pharmacol. Exp. Ther.* 252:875–79
79. Schenkman, J. B., Sligar, S. G., Cinti, D. L. 1981. Substrate interaction with Cytochrome P-450. *J. Pharmacol. Exp. Ther.* 12:43–71
80. Schenkman, J. B., Remmer, H., Estabrook, R. W. 1967. Spectral studies of drug interaction with hepatic microsomal cytochrome. *Mol. Pharmacol.* 3:113–23
81. Bayliss, W. M. 1901. On the local reactions of the arterial wall to changes of internal pressure. *J. Physiol.* 28:220–31
82. Kauser, K., Clark, J. E., Masters, B. S., Harder, D. R., Roman, R. J. 1990. Inhibitors of cytochrome P450 attenuate the myogenic response of dog renal arcuate arteries. *FASEB J.* 4(4):4911
83. Harder, D. R., Sanchez-Ferrer, C., Kauser, K., Stekiel, W. J., Rubanyi, G. M. 1989. Pressure releases a transferable endothelial contractile factor in cat cerebral arteries. *Circ. Res.* 65:193–98
84. Rubanyi, G. M., Vanhoutte, P. M. 1984. Hypoxia releases a vasoconstrictor substance from the canine vascular endothelium. *J. Physiol.* 364:45–56
85. Sylvester, J. T., McGowan, C. 1978. The effects of agents that bind to cytochrome P-450 on hypoxic pulmonary vasoconstriction. *Circ. Res.* 43: 429–37
86. Busse, R., Pohl, U., Kellner, C., Klemm, U. 1983. Endothelial cells are involved in the vasodilatory response to hypoxia. *Pflugers Arch.* 397:78–80
87. Jackson, W. F. 1988. Lipoxygenase inhibitors block O_2 responses of hamster cheek pouch arterioles. *Am. J. Physiol.* 255:H711–16
88. Cunningham, F. M., Woollard, P. M. 1987. 12(R)-Hydroxy-5,8,10,14-eicosatetraenoic acid is a chemoattractant for human polymorphonuclear leucocytes in vitro. *Prostaglandins* 34:71–78
89. Hammarstrom, S., Hamberg, M., Samuelsson, B., Duella, E. A., Stawiski, M., et al. 1975. Increased concentrations of nonesterified arachidonic acid, 12*L*-hydroxy-5,8,10,14-eicosatetraenoic acid, prostaglandin E_2, and prostaglandin $F_{2\alpha}$ in epidermis of psoriasis. *Proc. Natl. Acad. Sci. USA* 72: 5130–34
90. Rosolowsky, M., Falck, J. R., Willerson, J. T., Campbell, W. B. 1990: Synthesis of lipoxygenase and epoxygenase products of arachidonic acid by normal and stenosed canine coronary arteries. *Circ. Res.* 66:608–21
91. Mullane, K. M., Read, N., Salmon, J. A., Moncada, S. 1984. Role of leukocytes in acute myocardial infarction in anesthetized dogs: Relationship to myocardial salvage by anti-inflammatory drugs. *J. Pharmacol. Exp. Ther.* 228:510–22
92. Ma, Y-H., Harder, D. R., Clark, J. E., Roman, R. J. 1990. Effects of 12-hydroxyeicosatetraenoic acid (12-HETE) on dog renal arcuate arteries. *FASEB J.* 4(4):3361
93. Masferrer, J., Mullane, K. M. 1988. Modulation of vascular tone by 12(*R*)-, but not 12(*S*)-hydroxyeicosatetraenoic acid. *Eur. J. Pharmacol.* 151:487–90
94. Oliw, E. H. 1984. Metabolism of 5(6)-

epoxyeicosatrienoic acid by ram seminal vesicles formation of novel prostaglandin E_1 metabolites. *Biochim. Biophys. Acta* 793:408–15

95. Carroll, M. A., Schwartzman, M., Capdevila, J., Falck, J. R., McGiff, J. C. 1987. Vasoactivity of arachidonic acid epoxides. *Eur. J. Pharmacol.* 138:281–83
96. Ellis, E. F., Police, R. J., Yancey, L., McKinney, J. S., Amruthesh, S. C. 1990. Dilation of cerebral arterioles by cytochrome P-450 metabolites of arachidonic acid. *Am. J. Physiol.* 259: H1171–77
97. Lands, W. E. M. 1984. Biological consequences of fatty acid oxygenase reactions. *Prostaglandins Leukotrienes Med.* 13:35–46
98. Schwartzman, M. L., Falck, J. R., Yadagiri, P., Escalante, B. 1989. Metabolism of 20-hydroxyeicosatetraenoic acid by cyclooxygenase: Formation and identification of novel endothelium-dependent vasoconstrictor metabolites. *J. Biol. Chem.* 264:11658–62
99. Fitzpatrick, F., Ennis, M., Baze, M., Wynalda, M., McGee, J., et al. 1986. Inhibition of cyclooxygenase activity and platelet aggregation by epoxyeicosatrienoic acids: influence of stereochemistry. *J. Biol. Chem.* 261:15334–38
100. Garcia, M. P., McGiff, J. C., Carroll, M. A. 1990. Renal vascular effects of 5,6-epoxyeicosatrienoic acid (5,6-EET), a cytochrome P450-dependent arachidonate (AA) metabolite. *FASEB J.* 4(4):4221
101. Malik, K. U., McGiff, J. C. 1975. Modulation by prostaglandins of adrenergic transmission in the isolated perfused rabbit and rat kidney. *Circ. Res.* 36:599–609
102. Giesen, E. M., Imbs, J. L., Grima, M., Schmidt, M., Schwartz, J. 1984. Modulation of renal ATPase activities by cyclic AMP *Biochem. Biophys. Res. Commun.* 120:619–24
103. Douglas, J. G., Romero, M., Hopfer, U. 1990. Signaling mechanisms coupled to the angiotensin receptor of proximal tubular epithelium. *Kidney Int.* 38:S-43–S-47
104. Katz, A. I. 1985. Renal Na-K-ATPase: its role in tubular sodium and potassium transport. *Am. J. Physiol.* 242:F207–19
105. Postnov, Y. U., Reznikova, M., Boriskina, G. 1976. Na-K adenosine triphosphatase in the kidney of rats with renal hypertension and spontaneously hypertensive rats. *Pflugers Arch.* 362:95–99
106. Buchalew Jr., V. M., Puschett, J. B., Kintzel, J. E., Goldberg, M. 1969. Mechanism of exaggerated natriuresis in hypertensive man: Impaired sodium transport in the loop of Henle. *J. Clin. Invest.* 48:1007–16
107. Baldwin, D. S., Gombos, E. A., Chasis, H. 1965. Urinary concentrating mechanism in essential hypertension. *Am. J. Med.* 38:864–72
108. Guyton, A. C., Coleman, T. G., Cowley Jr., A. W., Scheel, K. W., Manning Jr., R. D., et al. 1972. Arterial pressure regulation. Overriding dominance of the kidneys in long-term regulation and in hypertension. *Am. J. Med.* 52:584–94
109. Abraham, N., Escalante, B., McGiff, J. C., Sacerdoti, D., Schwartzman, M., et al. 1988. Decreased formation of cytochrome P450 arachidonate metabolites prevents hypertension in the SHR. Counc. High Blood Pressure Res. *42nd Ann. Fall Conf. Sci. Sess.*
110. de Wardener, H. E., Clarkson, E. M. 1985. Concept of natriuretic hormone. *Physiol. Rev.* 65:658–759
111. McGiff, J. C., Quilley, C. P. 1981. The rat with spontaneous genetic hypertension is not a suitable model of human essential hypertension. *Circ. Res.* 48: 455–63
112. Beierwaltes, W. H., Arendshorst, W. J., Klemmer, P. J. 1982. Electrolyte and water balance in young spontaneously hypertensive rats. *Hypertension* 4:908–15
113. Dilley, J. R., Stier, C. T., Arendshorst, W. J. 1984. Abnormalities in glomerular function in rats developing spontaneous hypertension. *Am. J. Physiol.* 246:F12–20
114. Escalante, B., Falck, J. R., Yadagiri, P., Sun, L., Laniado-Schwartzman, M. 1988. 19(*S*)-hydroxyeicosatetraenoic acid is a potent stimulator of renal Na^+, K^+-ATPase. *Biochem. Biophys. Res. Commun.* 152:1269–74
115. Sacerdoti, D., Abraham, N. G., McGiff, J. C., Schwartzman, M. L. 1988. Renal cytochrome P-450-dependent metabolism of arachidonic acid in spontaneously hypertensive rats. *Biochem. Pharmacol.* 37:521–27
116. Kappas, A., Maines, M. D. 1976. Tin: a potent inducer of heme oxygenase in kidney. *Science* 192:60–62
117. Melby, J. C., Holbrook, M., Griffing, G. T., Johnston, O'N. 1987. Antihypertensive effects of an aromatase inhibitor in the spontaneously hypertensive rat. *Hypertension* 10:484–87