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Review article

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FUNCTIONAL ASPECTS OF EICOSANOID METABOLISM

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The sequential metabolism of eicosanoids by AA oxygenases confers biological properties that may have significant functional implications. Further-the interpretation of the effects of cyclooxygenase inhibitors on eicosanoid, dependent mechanisms needs to be reevaluated. Previously, if cyclooxygenase inhibitors affected the biological action of AA, it was considered to be due to elimination of a prostanoid-mediated component. This interpretation is no longer valid if a cell or tissue has significant P450 and cyclooxygenase activity. The cellular proximity of the P450-dependent monooxygenases and cyclooxygenase, described in tubules, interstitial cells, and blood vessels, may confer a unique ability of AA metabolites to coordinate tubular and vascular function.

Key Words: *Arachidonic acid, cytochrome p450, cyclooxygenase, vasoactivity.*

INTRODUCTION

The metabolism of arachidonic acid (AA) leads to a variety of eicosanoids with multiple and overlapping biological activities. It is now recognized that AA can be metabolized by three distinct biosynthetic pathways — cyclooxygenase, lipoxygenases and cytochrome P450 monooxygenases (P450) (*Fig. 1*). The generation of eicosanoids is initiated by the release of esterified AA from cellular lipids. Once liberated from membrane lipids by diverse stimuli, the free AA is rapidly metabolized. However, the pathway by which AA is transformed depends on the tissue, the kind of stimulus, cofactor availability and inhibitors, as well as disease states.

Cytochrome P450-dependent AA metabolism, formation of biologically active metabolites

The P450-dependent monooxygenases, mixed-function oxidase system strictly dependent on molecular oxygen and NADPH, can metabolize AA by three types of reactions: 1) allylic oxidation leading to the formation

of hydroxyeicosatetraenoic acids (HETEs); 2) olefin epoxidation leading to the formation of four different epoxyeicosatrienoic acids (5, 6; 8, 9; 12 and 14, 15; EETs) which can undergo hydrolysis by epoxide hydrolase to form the corresponding diol metabolites — the dihydroxyeicosatrienoic acids (DHETs), and 3) oxidation at the ω-and ω-1 positions to form the 20- and 19-HETEs, respectively (1, 2) (*Fig. 1*)

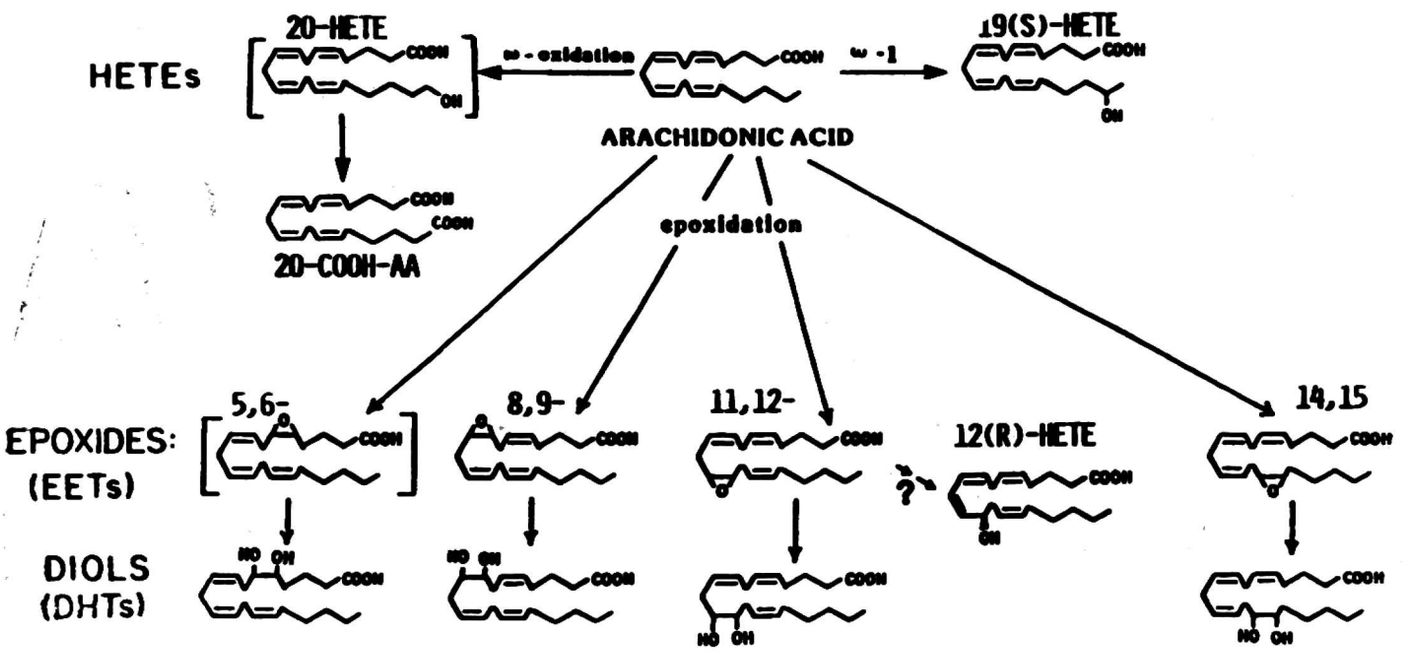

Figure 1: Arachidonic acid (AA) metabolism by cytochrome P-450-dependent monooxygenase to ω- and ω-1 hydroxyeicosatetraenoic acids (HETEs), epoxyeicosatrienoic acids (epoxides/EETs) [and dihydroxyeicosatetraenoic acids (diols/DHTs). Not shown are 8-, 9-, 11-, 12- and 15-HETEs, products of allylic oxidation.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

Within the kidney, regional variations in production of P450 AA metabolites have been reported by Takahashi et al. (3) based on the biosynthetic profile of cortical vs. medullary microsomal fractions. EETs are the principal P450-AA products of the renal cortex of the rat where they account for ca 60% of total P450-AA products, whereas in the medulla, 19- and 20-HETEs represent 90% of P450-AA metabolites generated by microsomal fractions. These findings on P450-AA product formation by the rat kidney differ from those reported by Schwartzman et al. (4) for the rabbit kidney in which large differences in ω- and ω-1 production of metabolites, when comparing cortex vs. medulla, were not observed. Quite unexpectedly, the specific activity of P450-AA metabolism was observed to be higher in the kidney than liver of the rabbit, with the highest activity three-fold that of the liver, in the outer medulla.

Using microdissection and cell isolation techniques, P450-AA metabolism has been studied in three discrete segments of the nephron, namely,

proximal, mTALH and collecting tubules. The major P450-AA metabolite formed by the S_1 segment of cultured rabbit proximal tubules is 5,6-EET. This epoxide is thought to mediate the natriuretic response to high dose angiotensin II in the S_1 segment (5), an effect of 5,6-EET that is cyclo-oxygenase-independent (5). We have isolated mTALH cells from rabbit outer medulla by centrifugal elutriation, a technique which provides mTALH cell fractions with less than 20% contamination by proximal and collecting tubular cells. Incubation of mTALH cells with radiolabeled AA results in the formation of P450-AA metabolites which resolve into two major peaks, P_1 and P_2, on reverse-phase HPLC (6). These peaks have characteristic biological activity, P_1 having vasodilator properties and P_2 inhibiting Na^+-K^+-ATPase activity (6, 7). Based on mass spectrometric analysis of P_1 and P_2, the principle eicosanoids in P_1 were identified as 19- and 20-HETEs, whereas P_2 contained predominantly 20-COOH-AA (8). Both 20-HETE and 20-COOH-AA, in addition to AA, inhibited Na^+ absorption (as measured by rubidium uptake) by mTALH tubules (8). The inhibitory effect of AA was unaffected by indomethacin, indicating that the inhibitory effects of P450-AA metabolites on Na^+ absorption in the epithelial cell of origin, mTALH, were independent of cyclooxygenase activity.

The third segment of the nephron, the rabbit cortical collecting tubules (9), where P450-dependent AA metabolism has been described, is a major target tissue for vasopressin. In this segment, epoxygenase metabolites have also been reported to be endogenous constituents. The DHETs, particularly 14-15-DHET, were shown to be potent inhibitors of the hydroosmotic effect of vasopressin, an effect mediated at a post-cAMP step (9).

It is becoming increasingly evident that in many tissues, for example, the kidney and the vasculature, several eicosanoids may be subject to further metabolism by oxygenases other than those giving rise to the initial product; and the expression of a particular biological action of these eicosanoids requires the cooperative interaction of different cell types containing different oxidative pathways. For example, 20-HETE, a principle product of AA metabolism by the medullary thick ascending limb (mTALH), inhibits Na^+ transport (8) in this segment and assumes vasoactivity (10) following transformation by cyclooxygenase of either renal interstitial cells or blood vessels.

The purpose of this review is to examine the evidence for further transformation of AA metabolites by other oxygenases — transcellular metabolism of eicosanoids — and to speculate on the (patho) physiological significance of this mechanism in terms of the control of blood pressure regulation.

Localization of renal and vascular AA oxygenases

There are differences in the profile of eicosanoids generated by zones and structures within the kidney. These differences are also evident in the segmental variation along the nephron. The structures which can metabolize AA via cyclooxygenase to prostaglandins are the collecting tubules (11), the glomeruli (12), the medullary interstitium (13) and the blood vessels (14). On the other hand, proximal convoluted tubules and mTALH have low or negligible cyclooxygenase activity (6, 14).

Lipoxygenase activity has been reported in rat glomeruli, in glomerular epithelial cells and in homogenized cortical tubules (15). However, the criteria for establishing the presence of lipoxygenase activity was not rigorous, as, for example, 12-HETE may originate from either a lipoxygenase or cytochrome P450-dependent monooxygenase activity. Like cyclooxygenase activity, P450 appears to be discretely localized, e. g., the mTALH and S_1 segment of the proximal tubule (5, 6) have a considerable capacity to generate P450-AA metabolites, but as noted, contain low cyclooxygenase activity. The cortical collecting ducts may occupy an intermediate position as they possess a considerable prostaglandin biosynthetic capacity and are also able to synthesize P450-AA metabolites (9).

Analogous to the segmental variation of the P450 pathway of AA metabolism within the nephron is the laminal variation within the vasculature where the highest activity of P450-dependent oxygenases and cyclooxygenase are found in the endothelium (16) A spectrum of P450-dependent mechanisms has been described within the vasculature, i e., blood vessels demonstrate a wide range of biosynthetic capacity and diversty with regard to P450-AA metabolites. Muscular arteries such as the femoral have the lowest representation and coronary arteries have among the highest, with the aorta intermediate (17). In response to stenosis, canine coronary arteries in addition to elevating production of prostaglandins demonstrate enhanced synthesis of EETs and their corresponding diols as well as 12- and 15-HETEs (18).

Transcellular Metabolism of Eicosanoids

Cytochrome P450-dependent products of AA metabolism are endowed with biological properties — vasoactivity and the ability to affect ion movement — that identify them as potentially important to blood pressure regulation (6). Cyclooxygenase activity in cell types — either vascular, mesenchymal or epithelial — in close proximity to cell types endowed with high P450 activity, may be critical to the local or regional expression of the full biological activity of certain P450-AA products. For example, the renomedullary interstitial cells, which have high cyclooxygenase

activity (13), and are adjacent to the mTALH, could be responsible for the further transformation of the P450 metabolites, 20-and 19-HETE, to vasoactive mediators.

Transcellular metabolism of two recently described vasoactive eicosanoids, 5,6-EET and 20-HETE generated intrarenally by P450 oxygenases, may be important to the expression of their vasoactivity (19, 20). The 5,6-EET and 20-HETE, each possessing natriuretic properties, require an additional step via cyclooxygenase, with formation of active metabolites, for expression of their vasoactivity at least in some vascular beds (19, 20). Thus, sequential metabolism of eicosanoids may link metabolic activity of a tissue/nephron segment to local blood flow.

5,6-EET; mechanism of vasoactivity

We have reported that the 5,6-EET was the only P450-AA epoxide to dilate the isolated perfused caudal artery of the rat (21). Removal of the endothelium decreased, by 70%, the vasodilator responses to 5,6-EET (*Fig. 2*). This finding indicates that the 5,6-EET was either transformed by the endothelium to a vasoactive metabolite or that it released an endothelial-dependent vasodilator. The endothelial-dependency was a function of the epoxide interacting with cyclooxygenase of the endothelium, as indomethacin (3μM) and aspirin (50μM) prevented the vasodilator response to 5,6-EET, while not affecting that to acetycholine (*Fig. 3*).

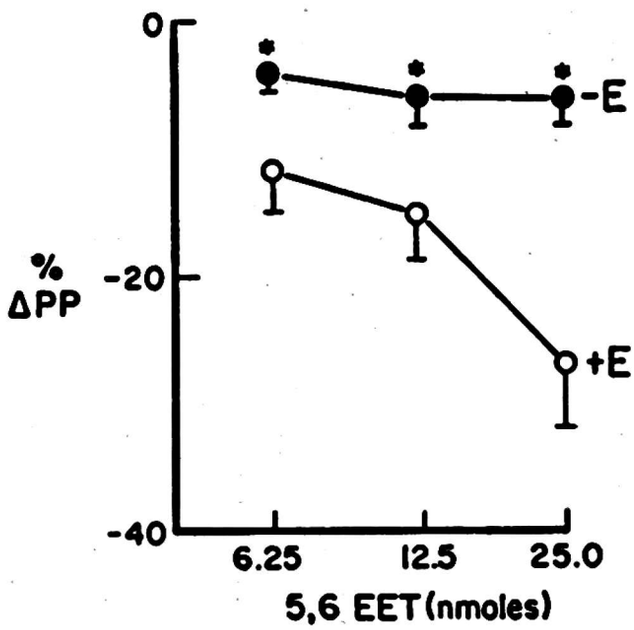

Fig. 2: Graph showing effect of deendothelialization on the vasodilator responses to 5,6-epoxyeicosatrienoic acid (5,6-EET). 5,6-EET (6.25—25 nmoles) was administered by bolus intra-arterial injection to the rat isolated, perfused caudal artery, preconstricted with phenylephrine. Results are expressed as percent change in perfusion pressure (PP) in the presence (o) and absence (●) of endothelium (E) ($n = 6$). *$p < 0.05$ compared with corresponding amount of 5,6-EET in the presence of endothelium.

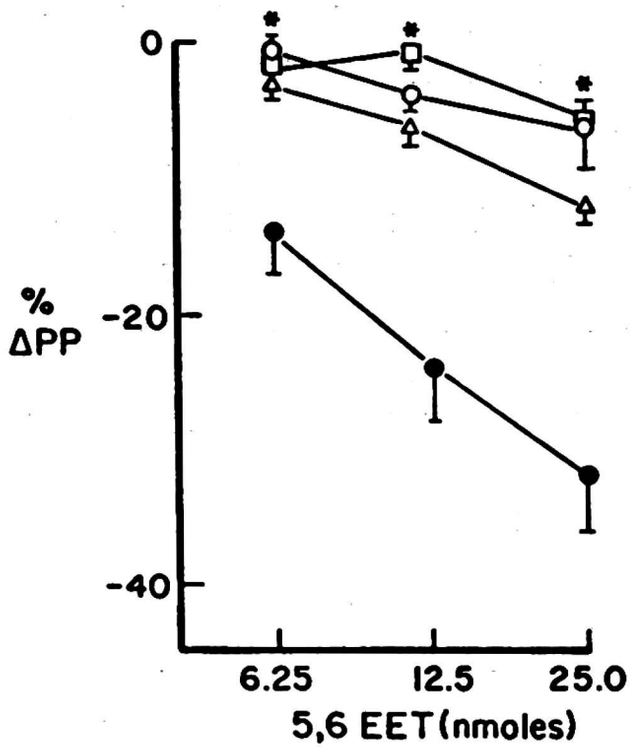

Figure 3: Graph showing effects of eicosatetraynoic acid (1 μM; n = 4) (□), indomethacin (3 μM; n = 7) (o), and aspirin (50 μM; n = 6) (△) on the vasodilator responses to 5,6-epoxyeicosatrienoic acid (5,6-EET; n = 17). Control values (●) represent the combined data from each inhibitor experiment. PP, perfusion pressure. *$p < 0.05$ compared with corresponding control value.

SKF525A (1.1 μM) and metyrapone (150 μM), inhibitors of type 1 and type 2 P450 activity, respectively, did not affect the responses to the 5,6-EET, indicating that 5,6-EET is not further metablized by P450 (19).

As the findings on the vasodilator mechanism of 5,6-EET are those of an indirectly acting vasodilator which is endothelial-dependent and either requires conversion by cyclooxygenase or releases a cyclooxygenase metabolite from endothelial cells, we measured vasodilator prostaglandins in the venous effluent. We did not observe any changes in the release of vasodilator prostaglandins (PGE_2 or PGI_2) from the caudal artery after the administration of 5,6-EET, suggesting that the vasodilator effect of the epoxide was independent of an indirect prostaglandin-mediated mechanism, i. e., through the release of endogenous AA. The cyclooxygenase dependent transformation products of 5,6-EET are, presumably, vasodilator analogs of PGI and PGE (22, 23). The intermediates, prostaglandin endoperoxide analogs, are transformed, or not, depending on tissue and species to analogs of PGE, PGF and PGI. 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. Upon 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, systemically.

Another mechanism for the expression of 5,6-EET vasoactivity has been proposed. In support of free radicals acting as the vasoactive species in the cerebral microcirculation, Ellis et al. (24) 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, as it was inhibited by either indomethacin or free radical scavengers (24). This study assumes physiological significance as 5,6-EET has been shown to constitute a significant portion of products synthesized from AA by brain slices (25).

The vasoactivity of 5,6-EET may be dependent on the specific vascular bed and species. The EETs, including 5,6-EET, have been reported to relax precontracted canine coronary arteries despite inhibition of cyclooxygenase (18). Moreover, the four EETs were equipotent in their coronary vasorelaxing ability, which findings stand in contrast to those in the caudal artery (21), the ductus arteriosus (26) and the intestinal (27) and cerebral microcirculations (24). In these vasculatures, 5,6-EET was the most potent vasodilator of the EETs; the other EETs were usually inactive. In the rat, 5,6-EET infused into the renal artery produced dose-dependent reductions in GFR and renal blood flow (3) whereas in the rabbit a renal vasodilator response to 5,6-EET occurred (10). 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 (3). In contrast, in the rabbit kidney, indomethacin eliminated the vasoactivity of 5,6-EET (10).

These apparent discrepancies may be reconciled on the basis of transformation of 5,6-EET (and 20-HETE, see below) via cyclooxygenase to the vasoconstrictor prostaglandin endoperoxides (5,6-oxido/epoxy-PGG_1 or PGH_1) in the rat whereas in the rabbit, the endoperoxide analog of PGH_1 is further transformed to PGI_1 or PGE_1 vasodilator analogs. These species differences are anticipated from the differences in renovascular responses to AA when comparing rat and rabbit. AA when injected into the rat kidney, gives rise to vasoconstrictor AA metabolites, presumably prostaglandin endoperoxides (28). In contrast, in the rabbit, injected AA dilates the renal vascular consequent to formation of vasodilator prostaglandins (29).

Vasoactivity of 20-HETE

20-HETE is a major metabolite of renal cortical and medullary microsomes (3, 4) and isolated mTALH cells (6). Like 5,6-EET, 20-HETE is a substrate for cyclooxygenase (30) and requires transformation by cyclooxygenase for expression of its vasoactivity. Following transcellular metabolism, the vascular effects of the cyclooxygenase metabolites arising from 20-HETE appear to be species and vascular bed dependent (as described above for AA metabolism).

The renal production of ω-hydroxylation metabolites, 19- and 20-HETE, is enhanced during the development of hypertension in spontaneously hypertensive rats (SHR). As 19 (S)-HETE has been reported to stimulate Na^+-K^+-ATPase from the rat renal cortex (31), and 20-HETE constricts isolated blood vessels (20), these AA metabolites may contribute to the development of hypertension. The contractile activity of 20-HETE on rat aortic rings was determined to be due to the formation of endoperoxide analogs of 20-HETE (30). The vasoconstriction was dose-dependent, and abolished by either indomethacin or the endoperoxide/thromboxane receptor antagonist, SQ29548 (20). However, rabbit mesenteric artery rings or the rabbit isolated perfused kidney, preconstricted with phenylephrine, respond to 20-HETE administration by vasodilation (7, 10). These responses, as those to AA, are presumably due to the formation of vasodilatory prostaglandin analogs of 20-HETE.

Transcellular metabolism of prostaglandins and other eicosanoids

Transformation of an eicosanoid by an additional oxygenase is not unique to 5,6-EET and 20-HETE. Cyclooxygenase products 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 cyclooxygenase metabolites, PGE_2 and PGF_2, can undergo metabolism by ω- and ω-1 hydroxylases (32) to metabolites having different biological activities and potencies than the parent prostaglandins (33). In addition, PGI_2 can be converted by P450 epoxygenases to 5,6-epoxy PGI_1 (34), a potent inhibitor of platelet aggregation (35). PGH_2 and 12(S)HETE, formed by platelets (36), may undergo transformation by endothelial cells and neutrophils to form PGI_2 (37) and 12,20--HETE (38), respectively. Thus, modification of the biological activity of prostaglandins and other eicosanoids by the P-450 system appears to be an important mechanism which assumes physiological significance when viewed in terms of alterations of P450 isozyme activity by changes

in hormonal status. For example, a greater than 100-fold induction of a pulmonary cytochrome P-450 isozyme, prostaglandin ω-hydroxylase, has been observed in pregnant rabbits (32).

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