20-HETE and the kidney: resolution of old problems and new beginnings

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McGiff, John C., and John Quilley. 20-HETE and the kidney: resolution of old problems and new beginnings. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R607–R623, 1999.—The protean properties of 20-hydroxyeicosatetraenoic acid (HETE), vasoactivity, mitogenicity, and modulation of transport in key nephron segments, serve as the basis for the essential roles of 20-HETE in the regulation of the renal circulation and electrolyte excretion and as a second messenger for endothelin-1 and mediator of selective renal effects of ANG II. Renal autoregulation and tubular glomerular feedback are mediated by 20-HETE through constriction of preglomerular arterioles, responses that are maintained by 20-HETE inhibition of calcium-activated potassium channels. 20-HETE modulates ion transport in the proximal tubules and the thick ascending limb by affecting the activities of Na+-K+-ATPase and the Na+-K+-2Cl− cotransporter, respectively. The range and diversity of activity of 20-HETE derives in large measure from COX-dependent transformation of 20-HETE to products affecting vasomotion and salt and water excretion. Nitric oxide (NO) exerts a negative modulatory effect on 20-HETE formation; inhibition of NO synthesis produces marked perturbation of renal function resulting from increased 20-HETE production. 20-HETE is an essential component of interactions involving several hormonal systems that have central roles in blood pressure homeostasis, including angiotensins, endothelins, NO, and cytokines. 20-HETE is the preeminent renal eicosanoid, overshadowing PGE2 and PGl2. This review is intended to provide evidence for the physiological roles for cytochrome P-450-derived eicosanoids, particularly 20-HETE, and seeks to extend this knowledge to a conceptual framework for overall cardiovascular function.

20-hydroxyeicosatetraenoic acid; cyclooxygenase; cytochrome P-450 monooxygenases; endothelin; nitric oxide; potassium channels; preglomerular microvessels; renal autoregulation; thick ascending limb; tubuloglomerular feedback; tumor necrosis factor

The fact that most of these substances are active in very low concentrations suggests that they have physiological functions, and many of those who work in this field must have indulged in wild private speculations based on this fact, but in most cases the evidence has been vague and the speculations have not been published. There can, however, be little doubt that some at least of these substances play fundamental physiological roles.

J. H. Gaddum (35)
modulates transport in key nephron segments (23); 20-HETE and a labile epoxide, 5,6-EET, are second messengers, accounting for many of the diverse actions of peptide hormones as mitogens, secretagogues, vasoactive agents, and regulators of volume and composition of body fluids (68, 70). We will review the role of 20-HETE and, to a lesser extent, 5,6-EET, in regulating the renal circulation and electrolyte excretion. There is a mushrooming literature on arachidonate epoxides that endorses one or more epoxides as candidate endothelially derived hyperpolarizing factors (9, 34). This area deserves separate consideration and will be considered only tangentially; it is the subject of a recent review (85).

Pandora’s box was opened by Capdevila and colleagues (10, 11) whose biochemical studies on CYP-dependent AA metabolism set the stage for the extraordinary findings related to the role of CYP-derived AA products in cardiovascular and renal regulatory mechanisms (87). The synthesis by Falck et al. (27) and by Ortiz de Montellano and Correia (76) of authentic standards of CYP-AA metabolites and specific inhibitors of CYP enzymes that spare COX and lipoxygenases was essential to the progression of studies (14), particularly as they relate to analysis of homeostatic mechanisms in terms of involvement of CYP-AA products. Selective inhibitors of \( \omega \)- and \( \omega-1 \)-hydroxylases that generate 20- and 19-HETEs, as well as epoxigenases generating 5,6-, 8,9-, 11,12-, and 14,15-EETs, are now available, which allows deletion of one or the other of the two major pathways of CYP-dependent AA metabolism (102). This capability is important in defining the functional contribution of either pathway, because the products of these pathways frequently have opposing effects on the circulation (11, 88). For example, the phase of rapid elevation of blood pressure in the spontaneously hypertensive rat (SHR) is associated with increased production of 20-HETE by \( \omega \)-hydroxylase (88) that is represented in renal tubules and blood vessels of the rat in the form of four isoforms of the CYP 4A family (50, 103). In contrast, a deficiency in epoxides, particularly 5,6-EET, rendered the rat liable to salt-induced elevations of blood pressure; namely, inhibition of epoxigenase activity made rats salt sensitive (69).

**THERE ARE DISTINCTIVE FEATURES OF CYP-RELATED AA METABOLISM COMPARED WITH COX- AND LIP oxyGENASE-DEPENDENT METABOLISM OF AA**

COX transformation of 20-HETE and 5,6-EET. The most extensively studied of CYP-AA metabolites in terms of transformation by COX is 20-HETE, the principal renal CYP-AA product (16, 44) (Fig. 1). It is converted by COX to a vasoconstrictor PGH2 analog (20-OH PGH2) that undergoes additional transformation by isomerases to vasodilator-diuretic metabolites (20-OH PGE2, 20-OH PGI2) and vasoconstrictor-antidiuretic metabolites [20-OH thromboxane (Tx) A2, 20-OH PGF2\(_{\alpha}\)] (79). COX-related metabolism has been proposed to couple changes in transport in tubular segments to local blood flow, effected by unmodified 20-HETE and by a vasoactive product of metabolism of 20-HETE by COX, respectively (70) (Fig. 2). For example, increased production of 20-HETE by the proximal tubules and the medullary thick ascending limb (mTAL), the principal sites of \( \omega \)-hydroxylase in the nephron, will reduce Na\(^+\) reabsorption in these neph-

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**Fig. 1. Arachidonic acid (AA) metabolism by cytochrome P-450-dependent monoxygenases to \( \omega \)- and \( \omega-1 \)-hydroxycosatetraenoic acids (HETEs), epoxyeicosatrienoic acids (epoxides, EETs) and dihydroxyeicosatetraenoic acids (diols, DHTs). 20-HETE and 5,6-EET can be converted by cyclooxygenase to analogs of prostaglandins.**

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Transcellular Metabolism of P450-AA metabolites

Fig. 2. Either 20-HETE or 5,6-EET generated by cytochrome P-450 (P450) system of nephron segments, on extrusion into renal interstitial, may be converted to vasoactive products by cyclooxygenase of adjacent structures such as blood vessels and interstitial cells. (With permission, from the Annual Review of Pharmacology and Toxicology, Volume 31, © 1991, by Annual Reviews, www.annualreviews.org (70.).)

ron segments by the action of the untransformed eicosanoid (23, 86). Subsequently, extrusion of 20-HETE contraluminally into the interstitial space will facilitate metabolism of 20-HETE by COX to vasoactive analogs such as 20-OH PGE₂ and 20-OH PGH₂ that can affect local blood flow. 20-HETE, like 5,6-EET (89), also has the ability to release prostaglandins as relaxation of the bovine coronary artery was related to stimulation of prostacyclin release from the artery (83). The subterminal HETEs, 16-, 18-, and 19-HETE, which are synthesized by the kidney, are also substrates for COX (14).

The 5,6 epoxide shows an extensive COX dependency that has three components (13), which vary with the vascular territory and the species: 1) 5,6-EET releases prostaglandins; 2) 5,6-EET is transformed by COX to vasoactive prostaglandin analogs; and 3) COX metabolism of 5,6-EET can generate reactive oxygen species, having vasoactive effects that may account for cerebral vasodilatation in the cat (21).

CYP-AA metabolism is readily manipulated. The level of activity of the CYP system can be increased by induction of CYP enzymes or decreased either by mechanism-based inhibitors of selective CYP enzymes or by inducing heme oxygenase, which degrades hemoproteins, including CYP (93). The flux of AA through the CYP system and generation of AA metabolites in the kidney are readily modifiable and can be related to hemodynamic and excretory function (81).

NO modulates activity of CYP enzymes. Nitric oxide (NO) exerts a tonic inhibitory influence on CYP metabolism of AA (55). Removal of the suppressant effects of NO by inhibiting NO synthase (NOS) will result in marked perturbations of renal function resulting from increased production of 20-HETE (97a, 98). Contrarily, increased production of NO will inhibit 20-HETE synthesis (2). These interactions operate at the renal tubular and vascular levels and are important in setting the level of activities of tubuloglomerular feedback (TFG), K⁺ channels, and renal vasomotion (79, 106, 118).

Isoforms of a CYP enzyme may differ in their functional effects. Each of the several isoforms of ω-hydroxylase in the rat kidney (4A1, -2, and -3) that metabolizes AA, generating 20-HETE and lesser quantities of 19-HETE (ω-1), differs in its primary localization, namely, tubules (50) vs. vasculature (103). These renal isoforms are subject to dissimilar regulatory influences such as, for example, testosterone and 4A2 (96), and differ with respect to catalytic efficiencies (73). Notable differences also exist amongst the several isoforms of the major enzymes in terms of their functional effects. For example, deficient production of 20-HETE by the isoform(s) localized in the mTAL has been postulated to be the basis of hypertension in the salt-sensitive Dahl/Rapp rat (65), inasmuch as 20-HETE, which modulates the Na⁺-K⁺-2Cl⁻ cotransporter in the mTAL, if deficient, will result in increased NaCl reabsorption (24). On the other hand, an opposite effect, elevation of vascular resistance in response to increased synthesis of 20-HETE in key segments of the preglomerular vasculature, may contribute to the development of hypertension in the SHR (88). The expression of the 4A2 isoform, a major isoform in renal microvessels, is several times greater in the rat male kidney than in the female kidney (50). A caveat should be inserted here: the 4A1 isoform of ω-hydroxylase, having the highest catalytic efficiency (13- to 40-fold more than 4A2 and 4A3) (73), although expressed at low levels in the kidney as detected by mRNA (50), may have effects on renal function equal to or greater than the other isoforms of ω-hydroxylases. Of the several 2C isoforms that catalyze NADPH-dependent metabolism of AA, producing primarily EETs, the 2C23 isoform has been identified as the major epoxygenase in the rat kidney and one responsive to increased salt intake (45). Moreover, 2C23 protein increased without detectable changes in gene transcription. Recent developments in the design of CYP inhibitors are directed toward targeting a specific isoform that will allow characterization of the functional effects of that isoform.

Storage of CYP-AA products. HETEs and EETs can be stored in tissue lipids (53) and released in response to hormonal stimuli as, for example, ANG II-stimulated, receptor-mediated hydrolysis of phospholipids (12). In contrast, prostaglandins are not stored, being synthesized on demand. Preformed HETEs and EETs, bound to lipids, represent a significant reservoir in those tissues in which they have been measured: the liver (53), kidney (12), and platelets (114). Additionally, membrane properties, as reflected in changes in permeability and activity of membrane-bound enzymes, can be altered by esterified CYP-AA metabolites (36). Because inhibitors of CYP enzymes do not prevent release of preformed CYP-AA products from lipid storage sites, the efficacy of inhibitors of CYP enzymes may be misinterpreted as being low. For example, despite adequate inhibition of CYP monooxygenase activity, ANG II released large quantities of HETEs from the Sn-2 position of the phospholipid, a site of storage of HETEs (12).

Receptors for CYP-AA products? CYP-AA products appear to act primarily within the cell of origin and, unlike most of the effects of prostaglandins, need not be extruded into the extracellular space to stimulate membrane receptors either on the cell of origin or nearby cells. However, a high affinity binding site for
14,15-EET was identified in membranes obtained from mononuclear cells (111). Unlike prostaglandins, CYP-AA products act primarily as autocrine rather than paracrine eicosanoids. Prostaglandins affect not only the cell of origin via stimulation of receptors coupled to G proteins but also contiguous cells and, in some instances, cells in remote sites (84). The ability of 11,12-EET to increase the activity of Ca\(^{2+}\) -activated K\(^+\) channels (K\(_{Ca}\)) in bovine coronary artery smooth muscle was reported to be dependent on the activation of a stimulatory G protein, an effect consistent with a conventional receptor-operated mechanism (60). However, involvement of a G protein in EET-induced activation of K\(_{Ca}\) could not be demonstrated in the bovine trachea; a direct effect of EETs was proposed, i.e., the K\(_{Ca}\) itself may be the receptor (20). This area is open and mostly unexplored and invites definitive studies.

**MYOGENIC RESPONSE OF BLOOD VESSELS AND AUTOREGULATION: 20-HETE MEDIATED**

Recent studies on CYP-AA metabolites have moved our understanding of vascular and transport mechanisms from the phenomenological to the mechanistic. This claim is abundantly evident in the exemplary studies of Harder and Roman (see Ref. 38), who have addressed autoregulation of the cerebral and renal circulations in terms of mediation by a CYP-derived AA product, 20-HETE. These studies defined an eicosanoid-dependent mechanism that linked the signals of increased pressure and stretch to contraction of blood vessels. The initial study addressed the contractile response of isolated blood vessels to elevations in transmural pressure, the myogenic response, and the basis for autoregulation of regional circulations (54). The myogenic response was first described by Bayliss (6) in 1901: “In the case of the kidney vessels, this reaction to increased tension is very marked.” Because the myogenic response was enhanced by the addition of AA in the face of COX inhibition and blocked by suppression of CYP-AA metabolism, an eicosanoid generated by the CYP pathway was suggested to be the mediator (38, 54, 64). The leading candidate was 20-HETE, on the basis of two indispensable findings: 1) the key arterial segments responsible for renal and cerebral autoregulation, the microvessels of each circulation, synthesized 20-HETE as their principal product, and 2) 20-HETE constricted these microvessels in nanomolar concentrations (49).

A provisional sequence for the mediation of renal (rat) and cerebral (cat) autoregulation, based on the recent studies of Harder and Roman (see Ref. 38), can be assembled: 1) pressure-induced entry of extracellular Ca\(^{2+}\) into vascular smooth muscle; 2) stimulation of phospholipase C (PLC), generating inositol 1,4,5-triphosphate (IP\(_3\)) and diacylglycerol (DAG); 3) lipase activation releases AA from DAG to be converted by \(\omega\)-hydroxylase to 20-HETE; 4) activation of protein kinase C (PKC) (cat cerebral) and tyrosine kinase (rat renal) by 20-HETE; and 5) species-dependent inhibition of K\(_{Ca}\) in vascular smooth muscle by either PKC or tyrosine kinase, resulting in a sustained vasoconstrictor action of 20-HETE.

A more detailed version of the steps involved in autoregulation follows. Increased pressure activates Ca\(^{2+}\) channels, resulting in a Ca\(^{2+}\) influx that depolarizes vascular smooth muscle and activates PLC, a Ca-dependent enzyme, with attendant generation of IP\(_3\) and DAG. DAG has the capacity to stimulate PKC, and may participate in activating Ca\(^{2+}\) channels. Furthermore, when acted on by diacylglycerol lipase, DAG releases AA for conversion by \(\omega\)-hydroxylase to 20-HETE (38). The contribution to the autoregulatory response of phospholipases A\(_2\) and D, if any, has not been examined. Although 20-HETE is a key component in the autoregulatory response in both cat cerebral and rat renal circulations, the next step differs in terms of mediation of the inhibitory effect of 20-HETE on K\(_{Ca}\) channels, a critical component of autoregulation; namely, 20-HETE activates PKC in the cat cerebral microvessels (58), whereas tyrosine kinase is activated by 20-HETE in the rat renal microvessels (98). 20-HETE also increases Ca\(^{2+}\) influx into smooth muscle of cat cerebral and possibly rat renal microvessels via L-type Ca\(^{2+}\) channels (35a). Beyond the capacity of 20-HETE to constrict those vascular segments responsible for autoregulation, 20-HETE is essential to the nonadapting property of autoregulation; that is, vasoconstriction is sustained despite the presence in vascular smooth muscle of K\(_{Ca}\) channels that when activated result in loss of vasoconstriction related to K\(^{+}\) efflux and decreased Ca\(^{2+}\) entry. The latter effects mediated by activating K\(_{Ca}\) channels are offset by the inhibitory effects of 20-HETE on K\(_{Ca}\) channels produced by either PKC (cat cerebral) or tyrosine kinase (rat renal), which result in reduced open-state probability of K\(_{Ca}\) channels (116). If K\(_{Ca}\) channels had been activated, membrane hyperpolarization would have resulted, preventing Ca\(^{2+}\) entry via voltage-sensitive Ca\(^{2+}\) channels and resulting in waning vasoconstriction. Decreased activity of K\(_{Ca}\) then underlies renal autoregulation; it is sustained as long as transmural pressure is elevated and is dependent on 20-HETE generation.

Inhibition of PKC in cat cerebral arteries converted 20-HETE-induced constriction to dilatation, providing additional evidence that PKC is an essential component of the signal transduction mechanism (58). Moreover, the myogenic response in cat cerebral arteries and arterioles involving activation of PKC with inhibition of K\(_{Ca}\) channels and activation of L-type Ca\(^{2+}\) channels was mimicked by 20-HETE. A PKC-dependent step may operate in most regional beds that have prominent myogenic responses to increased transmural pressure. As noted, PKC does not mediate the constrictor response to 20-HETE in the rat renal circulation and, by inference, is not involved in renal autoregulation in the rat. Rather, Sun et al. (98) have shown that 20-HETE activates the MAP kinase signal transduction pathway in rat renal arterioles, stimulating tyrosine kinase to reduce the activity of K\(^{+}\) channels. There is precedent for 20-HETE acting via the mitogen-activated protein kinase system, namely, in the mitogenic responses to...
epidermal growth factor in proximal tubules (61) and to norepinephrine in vascular smooth muscle (100).

The localization of ω-hydroxylase in the preglomerular microvessels, the segment of the renal vasculature primarily responsible for effecting changes in vascular resistance, and the biological properties of 20-HETE argue for its mediating renal autoregulation and, as will be addressed, its participation in TGF. This was put to the test by demonstrating that 17-octadecynoic acid (17-ODYA), an inhibitor of CYP-AA metabolism, abolished renal autoregulation (117). Furthermore, 17-ODYA has been shown to increase the activity of the large-conductance KCa channels in arterial smooth muscle, suggesting some degree of tonic inhibition by 20-HETE. The effect of 17-ODYA on KCa channels was reversed by 20-HETE, endorsing the concept that 20-HETE is an endogenous modulator of KCa channels and its activity were either lessened or eliminated (49).

In contrast, 20-HETE, in comparatively low concentrations (≤10 M), demonstrated vasoactivity when added to isolated blood vessels, because those factors that dissipated its activity were either lessened or eliminated (49).

The next step, to secure the proposed essential role of 20-HETE in autoregulation, on the basis of conventional analysis of a biological system, would have been to restore autoregulation, having deleted with an ω-hydroxylase inhibitor the putative mediator, by replacing endogenous 20-HETE with administration of authentic 20-HETE. However, this strategy will fail because 20-HETE is prevented from gaining access to its active site in sufficient concentration to produce sustained vasoconstriction by several factors, the first being avid binding to plasma proteins and the second being rapid uptake into tissue phospholipids to be stored until released by an appropriate stimulus, such as ANG II (12). In addition, free 20-HETE is metabolized by COX and probably by lipoxygenases (44). 20-HETE is also conjugated to glucuronide in the kidney, which presumably renders 20-HETE inactive. In humans, most of the 20-HETE (~90%) is excreted as the glucuronide conjugate (87), which does not appear to be the case in the rat. The convergence of these restricting factors determined the low vasoactive and natriuretic potency of 20-HETE when administered to the whole kidney (99). In contrast, 20-HETE, in comparatively low concentrations (10−9 M), demonstrated vasoactivity when added to isolated blood vessels, because those factors that dissipated its activity were either lessened or eliminated (49).

**TGF: 20-HETE MEDIATED**

The afferent glomerular arteriole, in addition to its contribution to renal autoregulation, is the effector limb of TGF that regulates glomerular filtration rate (GFR) by producing changes in preglomerular arteriolar resistance in response to soluteNaCl delivery to the mTAL. Solute delivery is monitored by the macula densa, which initiates the signal, resulting in constriction of the afferent arteriole, and thereby governs the regulation of extracellular fluid volume by determining delivery of solute to the renal tubules. TGF acts as “both sensor and effector of total salt and volume homeostasis” (40). 20-HETE is an essential component of TGF that may act on both limbs of the mechanism: the afferent limb, which is related to the signal initiated by the macula densa and its transmission, as well as the efferent limb, which is related to constriction of the preglomerular arterioles (118). Franco, Bell, and Navar (see Ref. 32) had anticipated that a non-COX, nonlipoxygenase AA metabolite was a component of TGF. They had reported that potentiation by AA of TGF was not affected by inhibition of COX or lipoxygenases, suggesting involvement of either AA directly, as reported for the effects of high concentrations of AA (~40 μM) on some ion channels (75), or a metabolite of AA generated by an undefined oxygenase. Zou et al. (118) examined the relationship of 20-HETE to TGF, prompted by studies indicating that 20-HETE constrains the afferent arteriole and is a principal product of AA metabolism in both the afferent glomerular arteriole and the mTAL, the latter sharing many properties with the contiguous macula densa. Potentiation of TGF in response to AA administration into the tubular fluid perfusing the loop of Henle and macula densa was blocked by inhibition of ω-hydroxylase with 17-ODYA and restored in the face of ω-hydroxylase blockade by the addition of 20-HETE to the tubular fluid. The experimental design of this study bypassed unavoidably the mechanism that transduced the tubular signal, increased solute delivery to the macula densa, to effect changes in afferent arteriolar tone. Increased 20-HETE levels within either the macula densa and/or the afferent arteriole may be related to elevation of cytosolic Ca2+. This was proposed by Bell and Navar (7) to be essential to the operation of TGF. Elevation of cytosolic Ca2+ in the macula densa may be linked to 20-HETE generation through activation of a Ca2+-dependent phospholipase that increases AA delivery to ω-hydroxylase. Indeed, PLA2 has been shown by Wang et al. (105) to be involved in mediating a Ca2+-related effect on apical K+ channels in rat mTAL via generation of 20-HETE. However, the rise in Ca2+ may also stimulate activity of neuronal nNOS, the constitutive NOS of the macula densa (109), which would attenuate TGF by NO suppression of 20-HETE production. In the study of Zou et al. (118), 20-HETE, having been introduced into the tubular fluid perfusing the macula densa, could diffuse across the luminal membrane of the macula densa and possibly pass through the mesangium to enter the afferent arteriole. However, 20-HETE is prey to metabolism by oxygenases and can also be catabolized, making the passage problematic. It appears unnecessary for 20-HETE of macula densa origin to gain “free passage” to the afferent arteriole, because that vascular segment generates 20-HETE (49). However, the question remains unanswered: is there a signal transmitted from the macula densa to the afferent arteriole that stimulates 20-HETE synthesis by the latter? It seems redundant to generate 20-HETE within both the macula densa and afferent arteriole unless metabolic and catabolic barriers are interposed, restricting diffusion of the eicosanoids. In any event, a major component in the mechanism underlying TGF has been identified: 20-HETE mediates changes in preglomerular arteriolar resis-
tance and perhaps participates in initiating the signal arising in the macula densa.

ANG II not only interacts with CYP-AA metabolites to modify ion transport in the mTAL and proximal tubules but also participates, via CYP-derived AA metabolites acting as second messengers, in the regulation of glomerular function by increasing the sensitivity of TGF (72), presumably through augmentation of 20-HETE production by preglomerular microvessels and possibly the macula densa (15). As ANG II increased 20-HETE synthesis by preglomerular microvessels, peptide-induced gain of TGF should occur. In addition, ANG II can increase 20-HETE production by the nephron, as has been shown for the mTAL, which is contiguous to the macula densa (63). The postglomerular efferent arteriole is also a target for ANG II, but has not been examined in terms of CYP-AA products acting as second messengers (92). Relative capacities of pre- and postglomerular microvessels to generate AA metabolites and/or respond to these metabolites differ greatly, as indicated by the functional effects of AA when applied to these blood vessels; namely, AA constricted the afferent arteriole but was without effect on the efferent arteriole (47).

NO, another potentially important component in TGF, can be integrated into this construct as a negative modulator of TGF in vivo (92). NO may be produced by both the macula densa and the functional effects of NO on TGF; namely, in response to dietary salt, NO blunted TGF (108). The negative modulatory effect of NO on TGF may be accounted for by suppression of 20-HETE synthesis. That is, production of NO by the macula densa and/or afferent arteriole, activated by the aforementioned increase in cytosolic Ca²⁺ in response to a luminal signal, can moderate constriction of the afferent arteriole by suppressing 20-HETE formation (2). In addition, the signal linking tubular fluid composition to changes in the afferent arteriolar tone may be suppressed by NO if the signal is related to a CYP product. Because 20-HETE constricts the afferent arteriole and possibly functions in the signal transduction pathway of TGF, deletion of 20-HETE by inhibiting ω-hydroxylase should inactivate TGF, which is the case (118). This construct is oversimplified inasmuch as it excludes several factors, such as adenosine and TxA₂, that can modify TGF (72). Nonetheless, the model contains key elements of TGF and should contribute to our understanding of abnormalities of TGF that have been observed in salt-sensitive Dahl/Rapp rats and in the SHR.

**TGF: INCREASED IN THE SHR; DECREASED IN SALT-SENSITIVE DAHL/RAPP RATS**

The general proposition is that high levels of 20-HETE augment and low levels of 20-HETE blunt TGF. In the SHR, TGF is enhanced during the developmental phase of hypertension, a period that coincides with the greatest activity of ω-hydroxylase and highest intrarenal levels of 20-HETE in the SHR (19, 88). Furthermore, the sensitivity of TGF is diminished in the adult SHR with established hypertension, when the high renal levels of 20-HETE subside. Additional support for regulation of TGF by NO-20-HETE interactions is provided by studies in Dahl/Rapp rats (108). High salt intake in the salt-resistant Dahl/Rapp rat blunted TGF through an NO-dependent mechanism, whereas inhibition of NOS prevented salt-induced attenuation of TGF. The latter effect is best explained by disinhibition of ω-hydroxylase on eliminating NO, thereby increasing 20-HETE production with augmentation of TGF. Enhancement of TGF produced by inhibition of NO synthesis during high salt intake did not occur in salt-sensitive Dahl/Rapp rats (108), a failure that can be accounted for by a deficiency in 20-HETE production in these rats. Deficient synthesis of 20-HETE by the mTAL is thought to be the underlying lesion in this hypertensive strain (65) and results in enhanced NaCl reabsorption by the mTAL, because 20-HETE modulates Na⁺ and Cl⁻ transport in this tubular segment (23).

**REGULATION OF GLOMERULAR AFFERENT ARTERIOLAR TONE**

The ability of preglomerular microvessels, particularly the afferent arteriole, to generate AA metabolites by the CYP pathway is directly related to the key role of these blood vessels in TGF and renal autoregulation. The afferent arteriole also generates AA products via both COX and lipoxygenase pathways, and each pathway can be activated selectively by vasoactive hormones. The complexity of hormonal interactions with oxygenases that metabolize AA in preglomerular microvessels is evident on reviewing the effects of ANG II on afferent arteriolar tone. ANG II constricted the rat arteriolar tone by stimulating PLA₂ to release AA that was transformed by lipoxygenases to vasoconstrictor AA products, 12- and 15-HETEs, the second messengers of the renal vasoconstrictor and other actions of ANG II (46). Inhibition of epoxygenases potentiated the constrictor response to ANG II, indicating that an EET, possibly 11,12-EET, counteracted ANG II-induced constriction of the afferent arteriole. This effect of ANG II on epoxygenase product formation in the rabbit has been related to an AT₂ receptor-mediated response (3). ANG II has also been shown to increase release of 20-HETE from rat preglomerular microvessels by stimulating an AT₂ receptor (15). However, in this study, isolated preglomerular microvessels were employed; these represent a mixture of arterial and arteriolar elements varying between 15 and 150 µM, whereas the studies that addressed vascular reactivity to ANG II made use of perfused glomerular arterioles attached to glomeruli, usually with tubular elements. Because vascular segments of various sizes, ranging from interlobular to terminal arterioles, differ in terms of reactivity, distribution of receptors, and in all probability oxygenase components, comparisons of results obtained from studies based on different renal vascular preparations are made with difficulty and then only with due respect for differences just cited. For example, stimulating PLC in response to increased transmural pressure in preglomerular microvessels elicited forma-
The concept of vascular segmentation as reflected in the differential distribution of receptors [endothelin A (ET_A) vs. AT_1 vs. AT_2], oxygenases, and their principal AA products along the lengths of blood vessels is useful for understanding segmental responsiveness of the renal vasculature under normal and diseased conditions (71a). For example, increased myogenic reactivity of the intermediate interlobular artery has been identified in the SHR, whereas the WKY rat does not demonstrate this abnormality (41). Furthermore, these difficulties are compounded when making interspecies comparisons. In defining the determinants of vascular reactivity to hormones, NO enters the schema once again by virtue of inhibiting 20-HETE synthesis. The greater sensitivity of the efferent arteriole to ANG II in the rabbit reflects the presence of NOS in the afferent arteriole and its absence from the efferent arteriole (51). A similar differential effect of ANG II on pre- and postglomerular vascular resistances occurs in the dog and, as in the rabbit, is determined by synthesis of NO by afferent but not efferent arterioles (92).

Norepinephrine also constricted the rat afferent arteriole but did so independently of stimulating PLA_2 and the activation of lipoxygenases (46). Norepinephrine-induced constriction was augmented by COX inhibition, suggesting that a vasodilator metabolite of AA, presumably PGE_2 and/or PGI_2, opposed the constriction, suggesting that a vasodilator metabolite of AA, induced constriction was augmented by COX inhibition of either NOS or COX but was inhibited by indomethacin, suggesting that formed products act as second messengers and modulators of vasoactive hormones and to AA administration (51, 72, 92).

General statements can be made on the basis of the above analysis regarding the renal vascular effects of hormones as modified by eicosanoids. Arachidonate metabolites act as second messengers and modulators of vasoactive hormones. Eicosanoid-hormonal interactions vary according to the vascular segment, experimental conditions, species, and sex. Within the vasculature, longitudinal variations in oxygenases and their products are evident, as is differential localization of CYP and COX in blood vessels viewed cross-sectionally, e.g., \( \omega \)-hydroxylase in vascular smooth muscle and eicosanoid epoxygenase in the endothelium. The interactions of vasoactive hormones and CYP-dependent AA metabolism show a high degree of selectivity relative to a given agonist acting on a particular segment of the renal vasculature and to the eicosanoid released by the metabolism of 20-HETE (38), whereas stimulation by ANG II of PLA_2 produced the lipoygenase products 12- and 15-HETE (46).
hormone. Findings based on isolated structures should be retested in an integrated experimental preparation in which the renal vasculature and tubules are represented. Observations made on isolated tissues, cultured cells, and cell homogenates are limited by the absence of paracrine and endocrine influences that, in situ, greatly affect the response of the cell or tissue. The relationship of an individual effect of a hormone on a renal cell or tissue to the resultant of the multiple effects of that hormone on renal function has been admirably summarized by Cogan (18) for ANG II; it applies to vasoactive hormones generally: “...the interconnected, dynamic control of circulatory and tubular transport processes is clearly important in the complex governance of renal function attributable to angiotensin II.” The operative words are “interconnected” and “governance” (18).

CYP ARACHIDONATE METABOLITES: TRANSFORMATION BY COX

Metabolism of CYP-AA products by COX generates metabolites that extend the range of activities of the parent compound. The renal effects of hyperchloremia demonstrated graphically the functional consequences resulting from transformation of CYP-AA metabolites by COX to prostaglandin analogs of 20-HETE (5). Chloride concentration studied over a range of 87–117 mM regulates the flux of AA products through COX-dependent pathways and determines the renal functional response to a given Cl− concentration (113). Vasodilator-natriuretic eicosanoids predominated when Cl− concentration was low (87 mM), and vasoconstrictor sodium-retaining eicosanoids predominated during hyperchloremia, defined as a Cl− concentration of 117 mM. Consequently, inhibition of COX produced renal vasodilatation and natriuresis under conditions of hyperchloremia and vasocostriction with Na+ retention at low Cl− concentrations. When Cl− concentration was normal (102 mM), COX inhibition did not affect renal blood flow or sodium excretion.

In the canine kidney, the renal functional response to hyperchloremia had been reported to be the consequence of increased renal TxA2 production (8). A TxA2-related mechanism could not be identified in the rat kidney, because inhibition of TxA2 synthase did not affect the renal functional response to hyperchloremia. In any event, inhibition of COX did prevent the negative effects of hyperchloremia on GFR and sodium excretion, suggesting involvement of a prostanooid-related mechanism. The endoperoxide PGH2, having renal functional effects indistinguishable from those of TxA2, was considered next to be a potential mediator of the response to hyperchloremia. Blockade of the TP receptor with SQ 29548 prevented the depression of GFR and Na+ excretion produced by hyperchloremia, advancing the candidacy of PGH2 as the mediator. However, a more selective antagonist of the TP receptor, BMS 180291, did not affect the renal response to hyperchloremia, eliminating PGH2 as the mediator. Two studies had been published indicating that a metabolite of 20-HETE generated by COX has properties similar to those of the eicosanoid mediator of hyperchloremia: 1) 20-HETE was converted by COX to vasoconstrictor prostaglandin analogs, 20-OH PGG2 and 20-OH PGH2 (59) and 2) the contractile action of 20-HETE on rat aorta was blocked by either inhibition of COX or antagonism of the TP receptor with SQ 29548 (26), duplicating the inhibitory effects of pharmacological blockade on the renal functional response to hyperchloremia. Moreover, 20-HETE is the principal product of AA metabolism in preglomerular microvessels (49).

This study challenges conclusions regarding the identification of putative lipid mediators based exclusively on pharmacological criteria. 20-HETE and the substrate of COX inhibition are both obligate for expression of the renal response to hyperchloremia. Because direct measurements of renal 20-OH PGH2 were not available, conclusions regarding its role as mediator must be conditional.

This study challenges conclusions regarding the identification of putative lipid mediators based exclusively on pharmacological criteria. 20-HETE and the subterminal HETEs, 16-, 18-, and 19-HETE, as well as 5,6-EEt, are substrates for COX that, as a first step, generate a PGH2 (endoperoxide) analog of the parent compound (14, 59). The PGH2 analogs of 20-HETE and 5,6-EEt have been shown to undergo additional conversion by prostaglandin isomerases to PGE2, TxA2, and PGl2 analogs that are biologically active (13, 79). The ability of an aspirin-like drug to inhibit a biological effect, therefore, does not always indicate that a prostaglandin is the responsible agent. Rather, a CYP product that requires transformation by COX to express a particular effect should be considered a potential mediator of the biological response under study: if the response is attenuated by inhibition of CYP-AA metabolism, an AA metabolite of the third pathway should be considered, and if in addition COX inhibition also attenuates the response, then a CYP-AA product that undergoes conversion by COX to an active metabolite is the presumptive mediator. There is an additional consideration relative to inhibition by aspirin-like drugs of the responses to CYP-AA products; namely, stimulation of prostanooid release by CYP-AA metabolites may occur without the requirement for conversion of the CYP-AA
metabolite to one or more prostaglandin analogs (13, 89).

Species differences can be decisive in determining the vascular responses to CYP-AA products. Unlike the vasoconstrictor effect of 20-HETE in the rat kidney (49), 20-HETE dilated the rabbit renal vasculature (16), an effect prevented by inhibition of COX and related to transformation of 20-HETE to a vaso dilator prostaglandin analog, most likely 20-OH PGE2 and/or 20-OH PGI2. This observation raises questions regarding the eicosanoid mediator of renal autoregulation in the rabbit, because 20-HETE is converted by COX to vaso dilator metabolites, which a priori would not be candidates for mediating renal autoregulation. However, findings based on exogenous 20-HETE and its transformation by COX may not be relevant to endogenous untransformed 20-HETE, which mediates renal autoregulation by acting intracellularly within preglomerular microvessels.

**RENAL TUBULAR TRANSPORT AND CYP-AA PRODUCTS**

We entered the area of CYP-AA metabolism by chance while following the trail of potential lipid modulators of Na\(^+\)-K\(^+\)-ATPase. Because mTAL cells possess an abundance of Na\(^+\)-K\(^+\)-ATPase, indicating the importance of this tubular segment in regulating transepithelial sodium movement, the daunting task of isolating mTAL cells in homogeneity was undertaken by Ferreri (then a graduate student) and colleagues (30). A study by Smith et al. (97), based on immunocytochemical evidence for detecting COX, indicated negligible capacity of the mTAL to generate prostaglandins. Nonetheless, isolated mTAL cells possessed a large capacity to metabolize AA via a non-COX pathway (30). A CYP-dependent metabolite of AA is probably involved, because the proximal tubules, also devoid of COX, were known through the work of Endou (22) to be heavily invested with CYP monooxygenase(s). Another feature of the mTAL is the coating of the cell surface with a glycoprotein (Tamm-Horsfall) that functions in an immunomodulator mechanism (43) involving tumor necrosis factor (TNF)-\(\alpha\) activation of the inducible form of COX (COX-2) (29).

Rabbit mTAL cells, when incubated with \(^{14}\)C-labeled AA, formed products that segregated into two peaks on the basis of their reverse-phase HPLC retention times (95). AA product formation by homogenates of mTAL cells required NADPH, a major criterion of the involvement of a CYP monooxygenase pathway. Peak heights were unaffected by inhibition of COX but were suppressed by inhibition of CYP. Because the major impetus for these studies on the mTAL was the identification of novel AA products that participate in the control of vasomotion and NaCl excretion, their biological properties were defined in terms of their ability to 1) relax precontracted arterial rings and 2) inhibit Na\(^+\)-K\(^+\)-ATPase. Peak I, the more polar peak, yielded vasoactive material that relaxed rabbit pulmonary artery rings, as well as material capable of inhibiting Na\(^+\)-K\(^+\)-ATPase (Fig. 3). Peak II was devoid of vasoactive substances, although a potent inhibitor of Na\(^+\)-K\(^+\)-ATPase activity was recovered from this peak.

The AA metabolites in peaks I and II were identified by gas chromatography-mass spectrometry; peak I contained two major components, 19- and 20-HETE (17). Peak II contained a single major component, 20-COOH.
AA, a metabolite of 20-HETE and, like 20-HETE, an inhibitor of transport. The importance of this study also derived from the localization of AA products to specific renal structures, in particular the mTAL, a key tubular segment that regulates salt and water metabolism. Heretofore, microsomes, homogenates, and subcellular fractions of either whole kidney or renal zones (cortex, outer and inner medulla) had been used to study renal CYP-AA metabolism.

When the effects of 20-HETE and 20-COOH-AA on ion fluxes in the mTAL were analyzed in terms of the principal site of action, they exhibited a furosemide-like effect on the Na⁺-K⁺-2Cl⁻ cotransporter (23, 24) and thereby secondarily diminished Na⁺ pump activity by limiting Na⁺ entry at the apical surface (Fig. 4). In contrast, in the proximal tubules, 20-HETE inhibited Na⁺-K⁺-ATPase by activating PKC, which in turn phosphorylated rat α Na⁺-K⁺-ATPase, decreasing enzyme activity and promoting Na⁺ excretion (74, 86). As 20-HETE and 20-COOH-AA have equal inhibitory actions on the mTAL cotransporter, there is a distinct possibility, heretofore unexamined, that 20-HETE requires transformation to 20-COOH-AA to inhibit the cotransporter.

In addition to modulating the cotransporter by a direct inhibitory effect, 20-HETE reduced the activity of the apical 70-pS K⁺ channel, thereby decreasing the activity of the cotransporter but in this instance doing so indirectly by virtue of the requirement of the functional integrity of the cotransporter for K⁺ entry, which is largely provided by recycling K⁺ via mTAL K⁺ channels (104) (Fig. 4). The basal activity of the 70-pS K⁺ channel was enhanced by blocking CYP-AA metabolism with 17-ODYA, suggesting tonic inhibition of channel activity by sustained synthesis of 20-HETE. Like 17-ODYA, NO suppresses ω-hydroxylase. Prevention of NO formation by the mTAL, therefore, should decrease the activity of the 70-pS K⁺ channel (62) by disinhibiting ω-hydroxylase, resulting in increased 20-HETE synthesis. Conversely, the ability of NO to increase the activity of the 70-pS K⁺ channel via a cGMP-dependent pathway may be related to NO suppression of 20-HETE synthesis (62).

The activity of ANG II on the 70-pS K⁺ channel was shown to have an NO component that was elicited by high concentrations of the peptide. ANG II had biphasic effects on 70-pS K⁺ channel activity that were concentration-dependent and accounted for by changes in 20-HETE production (63). At low concentrations (50 pM) ANG II inhibited the 70-pS K⁺ channel by stimulating production of 20-HETE, whereas at high concentrations (50 nM) ANG II stimulated production of NO that in turn suppressed synthesis of 20-HETE, thereby activating the K⁺ channel by removing an inhibitory modulator. Interactions of NO with ω-hydroxylase are also evident at the organ and whole animal level. Thus the marked suppression of renal function that follows inhibition of NO synthesis can be resolved primarily in terms of increased renal production of 20-HETE, having removed the inhibitory influence of NO (79). Sequel to the physiological studies are underway that address the possible contribution of CYP-AA metabolites to diseases in terms of either excess or deficient production. The “first returns are in,” and, as in the physiological studies, gaps in our understanding of the pathophysiology of human disease have been filled, for example, the renal functional abnormalities that occur in Hepatorenal Syndrome, a terminal complication of hepatic cirrhosis (87). Patients with advanced cirrhosis excreted large quantities of 20-HETE that exceeded urinary excretion of prostaglandins and thromboxane by several fold. In hepatic cirrhosis, production of 20-HETE by the preglomerular vasculature has been proposed to be the basis of the progressive deterioration of renal function produced by the intense renal vasoconstriction that occurs in the absence of either renal histological or morphological changes.

**mTAL-TNF-COX-2 AND SHOCK**

The renal functional consequences of enhanced production of 20-HETE in hepatic cirrhosis lead to a consideration of the reverse, diminished production of 20-HETE as a key factor in the development of circulatory shock. TNF and NO are the prime movers in shock, acting through altered activity of COX and CYP. In response to either lipopolysaccharide (LPS) or ANG II, the mTAL produced TNF (25, 29). A causal relationship between changes in ion flux and TNF production in the mTAL was identified; TNF antisera prevented the effects of TNF on §Rb uptake, an index of K⁺ movement (25). However, TNF-induced changes in §Rb uptake in the mTAL were inhibited by indomethacin, not, as expected, by inhibition of CYP-related AA metabolism, suggesting that a prostaglandin-dependent rather than a CYP-related mechanism modulated ion movement in the mTAL in response to expression of TNF. That is, expression of TNF in the mTAL caused AA to be metabolized by COX-2 (28, 66), which partially accounts for TNF-induced diuresis (101). TNF increased release of PGE₂ from the mTAL after a latent period of more than 4 h, associated with accumulation of COX-2.
mRNA (25). The interfacing of TNF via NO (or its product, peroxynitrite) with these oxygenases, causing expression of COX-2 and suppression of CYP activity, is likely but lacks definitive evidence to be integrated into an operational concept of mTAL transport.

These findings, based on studies of isolated cells, are important for understanding responses of intact animals to systemic challenges. For example, TNF production was suggested to be a key component in a blood pressure buffering mechanism that opposes the pressor effect of ANG II (31). Infusion of ANG II for 10 days increased production of TFN and PGE$_2$ by more than threefold in mTAL tubules isolated from the rat kidney on the 10th day compared with those obtained from rats receiving solvent. The pressor response of the rat to infusion of ANG II was exacerbated when TNF antisera was administered; mean arterial blood pressure increased by more than 20 mmHg. The findings in isolated mTAL tubules regarding interactions of TNF and eicosanoids in response to ANG II stimulation, therefore, are translatable to a larger context, the whole animal.

These studies established the framework for inclusion of TNF and COX-2 as major components in the pathophysiology of septic and hemorrhagic shock (25) associated with suppression of CYP-AA metabolism. High circulating levels of TNF in shock cause a cascade of events involving increased NO production and enhanced prostaglandin synthesis (112, 115). Less well appreciated is the negative effect of NO on CYP activity; these mechanisms as are G proteins, signaling molecules and modulators are as essential to the operation of vasomotion and transepithelial ion movement. Furthermore, the generation of lipid mediators that mediate increased RVR in response to prostaglandin analogs and/or 20-OH TxA$_2$, whereby altering the balance of the principal eicosanoids: PGH$_2$; 20-HETE, the mTAL, then, can serve as a microcosm in which to study the principal components, the interactions of which in the systemic circulation govern the evolution of the pathophysiology of shock. In developing a comprehensive operational concept of shock, an essential component, diminished production of CYP-AA metabolites, particularly 20-HETE, must be included.

These studies support a general conclusion, namely, that acyl hydrolases, oxygenases, and their eicosanoid products are key components in regulatory mechanisms that affect vasomotion and transepithelial ion movement. Furthermore, the generation of lipid mediators and modulators are as essential to the operation of these mechanisms as are G proteins, signaling molecules, and protein kinases. In contrast to prostaglandins, which signal through G protein-coupled receptors and nuclear transcription factor peroxisome proliferator activated receptor-γ, the molecular basis for signaling by CYP-AA products awaits definition. The species of eicosanoid involved in cellular regulatory mechanisms may not be fixed, as has been shown in the mTAL after challenge with either LPS or ANG II, each activating a mechanism that depends on their ability to stimulate production of TNF by the mTAL associated with expression of COX-2 and suppression of CYP-AA metabolism (25, 28, 29).

**NO TONICALLY INHIBITS 20-HETE SYNTHESIS**

NO inhibits CYP initially by forming an iron-nitroxyristox complex at the catalytic unit of CYP (110) followed by time-dependent irreversible inactivation of CYP. These negative effects of NO on CYP-AA metabolism are in accord with observations regarding endotoxin (LPS)-induced suppression of hepatic CYP-dependent drug metabolism: \( \text{LPS} \rightarrow (+)\text{NOS} \rightarrow \text{NO} \rightarrow (-)\text{CYP metabolites} \). The renal functional consequences of inhibiting NOS on CYP-AA metabolism present a striking demonstration of the importance of NO as a tonic modulator of ω-hydroxylase activity. When ω-hydroxylase was derepressed by inhibiting NO synthesis, a surge in synthesis of 20-HETE occurred that increased mean aortic blood pressure, elevated renal vascular resistance (RVR), decreased GFR, and produced diuresis-natriuresis (79). Although endothelins had been shown in earlier studies to contribute to the acute elevation in blood pressure in response to inhibition of NO synthesis (90), the linkage to 20-HETE production had yet to be identified. Endothelin-1 (ET-1) initiated the cascade of events surrounding increased 20-HETE synthesis and subsequent metabolism of 20-HETE by COX to form prostaglandin analogs that constricted the renal vasculature and decreased GFR while promoting salt and water excretion (79) (Fig. 5). Based on these findings, the following interventions were selected to modify the renal response to inhibition of NO synthesis: 1) blockade of ET$_A$, because their activation stimulates lipases to release AA for metabolism by ω-hydroxylase; 2) inhibition of ω-hydroxylase; 3) inhibition of COX, which transforms 20-HETE into prostaglandin analogs; and 4) blockade of TP receptors that mediate increased RVR in response to prostaglandin analogs of 20-HETE. Each of these interventions restored to normal the renal functional abnormalities initiated by removal of the inhibitory effect of NO on ω-hydroxylase activity. Because blockade of the TP receptor attenuated the increased RVR but left unaffected the natriuresis, the likely mediators of renal vasoconstriction were 20-OH PGH$_2$ and/or 20-OH TxA$_2$, whereas other prostaglandin analogs of 20-HETE were responsible for the natriuresis, probably 20-OH PGE$_2$ and/or 20-OH PG$_1$ (Fig. 5). This study underscores the importance of 20-HETE to the circulatory and renal functional effects of endothelins and supports the proposal that 20-HETE is a second messenger for ET-1 (77).

**20-HETE: SECOND MESSENGER FOR ET-1?**

Analysis of the renal functional effects of ET-1 in terms of eicosanoid dependencies (80) disclosed critical linkages in the anesthetized rat. 1) Vasodilator prostaglandins may contribute to the maintenance of GFR
and oppose ET-1 depression of GFR, because indomethacin potentiated the negative effects of ET-1 on GFR; alternatively, the effect of indomethacin may be accounted for by prevention of the generation of vasodilator prostaglandin analogs of 20-HETE (78). 2) The negative action of ET-1 on GFR and the diuretic-natriuretic effect of ET-1 were prevented by inhibition of CYP-dependent AA metabolism, indicating that a CYP-AA product mediated in large part these effects of ET-1. ET-1-induced increase in sodium excretion had been ascribed to a pressure natriuresis (56). Natriuresis, however, was independent of the pressor effect of ET-1 (39, 80). Moreover, ET-1 has been shown to be synthesized by the nephron and to have a direct effect on tubular reabsorption of Na⁺ (82). The identity of the renal functional effects of ET-1 and 20-HETE, natriuresis despite renal vasoconstriction and depression of GFR, gives additional support to the proposed role of 20-HETE as second messenger for the renal effects of ET-1.

**ET-1 AND 20-HETE IN DOCA-SALT HYPERTENSION**

The uninephrectomized rat, given excess salt and treated with DOCA (UNx/salt/DOCA) is characterized by vascular, cardiac, and renal hypertrophy resulting from the mitogenic effects of ET-1, which is increased in this model (90). An essential link has been established by Oyekan et al. (78) between these effects of ET-1, as well as those on blood pressure elevation and proteinuria, and increased production of 20-HETE acting as second messenger for ET-1. Blood pressure and protein excretion increased progressively over the 21-day period of the study associated with cardiac, renal, and vascular hypertrophy. Between the second and third weeks, urinary excretion of ET-1 and 20-HETE had increased by three- to fourfold. CoCl₂ treatment ameliorated the hypertension and proteinuria, prevented cardiac and renal hypertrophy, and reduced the increase in media-to-lumen ratio of renal arteries. CoCl₂ depletes CYP enzymes by inducing heme oxygenase, which accelerates heme degradation, including those of hemoproteins such as CYP, thereby impairing CYP-dependent AA metabolism (67). Blockade of the ETₐ receptor also lowered blood pressure and attenuated organ hypertrophy and proteinuria while decreasing excretion of 20-HETE. Additional evidence that 20-HETE mediated the mitogenic response to ET-1 was provided by observing the effects of ET-1 on aortic rings pulsed with [³H]thymidine before and after dibromodec-11-enolic acid (DBDD) inhibition of ω-hydroxylase activity. DBDD attenuated ET-1-induced increase in [³H]thymidine incorporation while not affecting that induced by 20-HETE (78).

CoCl₂ induction of heme oxygenase also increases formation of carbon monoxide (CO), a product of heme conversion to biliverdin (1). This action of CoCl₂ makes the interpretation of the effect of CoCl₂ problematic, because CO generation could be as important as CYP suppression to the capacity of CoCl₂ to ameliorate the pathophysiologic response in the UNx/salt/DOCA rat. Thus CO is vasodepressor and antiproliferative both through direct effects as well as by modifying the activity of heme-containing enzymes, including COX and CYP (52, 57). Like NO, CO can stimulate guanylate cyclase to produce vasodilation (91). Therefore, attribution of the beneficial effects of CoCl₂ solely to inhibition of 20-HETE synthesis may be incorrect, because this interpretation ignores the potential contribution of CO to reversal of the deleterious cardiovascular and renal effects of DOCA-salt treatment. To address this issue, UNx/salt/DOCA rats were treated with 1-amino-benzotriazole (ABT), an inhibitor of CYP activity that is thought to spare heme oxygenases (71). ABT did reduce the elevated blood pressure, as did CoCl₂. Unlike CoCl₂, ABT did not prevent organ hypertrophy and proteinuria, suggesting that CoCl₂-induced formation of CO contributed to the reduction in cardiovascular and renal hypertrophy and reversal of renal injury.

The ramifications of the functional consequences of CYP-AA products and CYP regulatory factors such as heme oxygenase that define the level of activity of the CYP isozymes seem endless. The functional impact of activation of heme oxygenases results not only in diminished CYP-AA product formation, but perhaps more importantly in generation of CO. The initial reports suggest that CO holds great promise for understanding regulatory mechanisms affecting the central
nervous system and renal and cardiovascular function. A fascinating interplay of heme oxygenase-CO, NO, endothelin, CYP, and COX products has been identified by Ceccani and colleagues (17a, 17b) as pivotal to controlling patency of the ductus arteriosus. Is the scientific world ready for another gas? Ready or not.

Perspectives

Have expectations and projections surpassed the hard facts obtained in studies on the CYP pathway of AA metabolism? Has fantasy eclipsed reality? We don’t think so. This field has been in flux, reflecting the uncertainties of a terra incognita, and is now in a period of consolidation in which assignment of key roles to CYP-derived arachidonate products rests on firm data. The case is compelling for mediation of renal and cerebral autoregulation by 20-HETE acting on vascular segments that determine changes in resistance in these regional circulations by decreasing the activity of K_{at} channels in vascular smooth muscle. The case is equally compelling for 20-HETE serving as the principal modulator of the Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter in the mTAL and as an essential component of TGF; 20-HETE also participates in the regulation of transport in the proximal tubules, probably by affecting Na\(^+\)-K\(^+\)-ATPase activity. Temporal relationships (short-term vs. long-term effects) must be considered, inasmuch as the immediate effect (in minutes) of ANG II on the mTAL is release of 20-HETE, whereas the long-term effect (in hours) is induction of COX-2 via stimulation of TNF-\(\alpha\) production. Species differences may be decisive; most of the studies were conducted in the rat and may not translate directly to rabbit, human, etc. Examination of the impact of dietary factors on CYP isoform activity is a fertile area for future studies.

There are pieces that thus far have resisted integration into developing schema, e.g., 20-COOH-AA, the dicarboxylic (dioic) product of 20-HETE that, like 20-HETE, inhibits the cotransporter but is devoid of vasoactivity. Some of the effects ascribed to 20-HETE in the nephron may result from its oxidation product, 20-COOH-AA, which is found in relative abundance in the mTAL. Similarly, 19(S)-HETE produced together with 20-HETE by several of the 4A isoforms has properties consistent with its acting in opposition to 20-HETE in transporting epithelia. Epoxides, particularly 11,12- and 5,6-EET, may also act in mechanisms that oppose the effects of 20-HETE in critical sites such as afferent glomerular arterioles. A persuasive case has also been made for the inclusion of NO and COX in the schema that comprehends the renal vascular and tubular actions of 20-HETE: namely, NO tonically inhibits \(\omega\)-hydroxylase, whereas COX extends the reach and range of 20-HETE after its conversion to prostaglandin analogs that produce a spectrum of biological responses. Another gas, CO, a product of heme oxygenase that degrades CYP enzymes and other hemoproteins (Heme \(\rightarrow\) CO + biliverdin + Fe), exerts a tonic inhibitory effect, as does NO, on \(\omega\)-hydroxylase activity and participates in the regulation of vascular tone and tubular transport. Sorting out the separate and overlapping spheres of activity of NO and CO will not be a simple task.

It is now evident that 20-HETE is the dominant renal eicosanoid overshadowing PGE\(_2\). Definition of the localization, catalytic efficiency and regulation of the several isoforms of the 4A family (1, 2, and 3) that metabolize AA in the rat kidney are essential, because recognition of their separate and overlapping spheres of activity should rationalize the apparent contradictory effects of 20-HETE, as for example on blood pressure. Namely, 20-HETE deficiency in the mTAL is associated with the development of hypertension in the salt-sensitive Dahl rat, whereas increased production of 20-HETE by the renal vasculature in the young SHR is associated with elevation of blood pressure. It is here that the application of molecular biological methods to resolve this and other issues in this research area has great promise. The high homology among the 20-HETE-forming CYP4A isoforms and the ability of many CYP isoforms to use AA as substrate and produce an array of EETs and \(\omega\)-1-, \(\omega\)-2-, and \(\omega\)-3-hydroxylated products pose problems in evaluating the physiological significance of each metabolite. The use of standard pharmacological manipulations (enzyme inhibitors, inducers, and antibodies) often leads to misinterpretation. However, with the growth in knowledge of the molecular characteristics of CYP isoforms, including gene sequences, structures, and chromosomal localization, the door is open to pharmacogenetic manipulation that is more specific. Still, much is to be done with regard to the molecular and cellular characteristics of each isoform, including tissue and cell-specific expression, promoter regulation, and relative catalytic efficiency with regard to AA. In vitro studies, using gene transfer technology to produce cells to overexpress these proteins, offer a means for evaluating the role of a specific metabolite. The use of specific antisense oligonucleotides administered with liposomes or with the help of viral vectors provides a better approach for targeted inhibition of CYP isoforms than do enzyme inhibitors. Techniques such as tissue-specific expression of a transgene, as well as inducible expression, may prove to be better than the gene knock-out mouse in identifying the effects of single isoforms. Finally, cosegregation of genotypes with phenotypes (e.g., hypertension) and eventually genetic linkage analysis in humans should shed light on the pathophysiological importance of these genes. This area of research is in the logarithmic phase of its development, offering boundless opportunities to biological scientists. The challenges are great, the intellectual rewards greater.

The most important thing is not any one particular piece but finding enough pieces and enough connections between them to recognize the whole picture.

Christian Nüsslein-Volhard (74a)
Michal Schwartzman for establishing the biochemical and molecular biological foundations and for critical commentary, to Dr. Nader Abraham for introducing us to this area of research, and to Drs. Nicholas Ferreri, Mairead Carroll, Adebayo Oyekan, and David Sacerditi for general contributions to the many faces of these studies by providing vigorous interdisciplinary approaches to this field.

This review was made possible by grants from the National Heart, Lung, and Blood Institute (34300 and 25394 to J. C. McGiff; 49275 to J. Quilley) and by American Heart Association Grant 940–318 (to J. Quilley).

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