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THE EICOSANOID FACTOR:
A DETERMINANT OF INDIVIDUALITY OF NEPHRON SEGMENTS

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Background: Nephron function is segmented; each segment has characteristic transport mechanisms and individual eicosanoid profiles. The transport function of the medullary thick ascending limb of Henle's loop (mTAL) establishes the osmolar gradient upon which extra cellular fluid volume (ECFV) conservation depends. The overriding importance of the mTAL to regulation of ECFV is evident in the diuretic-natriuretic potency of furosemide-like agents which target the mTAL.

Results: The mTAL has been shown to be heavily invested with cytochrome P450 monooxygenases (CYP), chiefly \( \omega-1 \) hydroxylase activity, that generate 19- and 20-hydroxyeicosatetraenoic acid (HETEs). However, displacement of \( \omega \) hydroxylase by an inducible cyclooxygenase mechanism (COX-2) can be effected by several interventions: long-term infusion of angiotensin II (ANG II), adrenalectomy and elevated extracellular Ca\(^{2+}\) concentrations. This switching mechanism (CYP \( \rightarrow \) COX-2) has been shown to be dependent on activation of tumor necrosis factor \( \alpha \) (TNF\(\alpha\)) by ANG II. It represents a long-term adaptive mechanism of the mTAL with production of PGE, whereas in the short-term, ANG II increases 20-HETE synthesis by the mTAL. The effect of Ca\(^{2+}\) on mTAL eicosanoid-related mechanisms provides an explanation for the natriuretic response to hypercalcemia and diminished ability to concentrate urine.

Conclusion: The expression of COX-2 in the TAL has been linked to activation of the renin-angiotensin system, glucocorticoid deficiency and hypercalcemia, all of which operate through a mechanism in which production of TNF\(\alpha\) by the TAL is pivotal.

Key Words: ANG II (Angiotensin II), Ca (Calcium), COX (cyclooxygenase), CYP (cytochrome P450 monooxygenases), TAL (thick ascending limb), 20-HETE (20-hydroxyeicosatetraenoic acid)
INTRODUCTION

Until recently, the role of lipid mediators and modulators in regulating renal function was restricted to a consideration of which prostaglandin operates in what mechanism that affects which structure: tubules, blood vessels or glomerulus. Mapping the renal distribution of cyclooxygenase (COX) disclosed a heavy concentration in the collecting ducts and the renal vasculature, including its glomerular representation, the sites identified by immunocytochemistry as most heavily invested with COX (1,2). The nephron, in particular, demonstrated a discontinuous expression of COX, viz., collecting ducts, mainly medullary, and the thin limbs of Henle's loop. However, two segments which account for the bulk of salt and water reabsorption, the proximal convoluted tubules and the thick ascending limb (TAL), were either conspicuously devoid of COX (as detected by immunocytochemistry) or negligibly endowed with the capacity to generate prostaglandins (1,2). We have examined these segments in terms of an alternative pathway of arachidonic acid (AA) metabolism, the cytochrome P450 monoxygenases (CYP), which has resulted in "opening up" the TAL to a detailed analysis of the diverse functional and pathophysiological consequences of normal and altered production of CYP-AA products (3,4). As the TAL occupies a key position in the regulation of extracellular fluid volume (ECFV), these studies are vital to understanding renal excretory function. The TAL establishes the osmotic gradient that defines the ability of the kidney to excrete either a concentrated or dilute urine and houses the Na⁺-K⁺-2Cl⁻ cotransporter, the target enzyme of the most potent diuretic agents, the loop diuretics (5).

The CYP pathway in the TAL

The initial study, the one that struck gold, cast us in the roles of Princes of Serendip as we, for the wrong reasons, and guided by the wrong star, fell upon a treasure trove of CYP-dependent arachidonate-related mechanisms (3). This study benefited from the collaboration of a colleague, N. Abraham, who advised us that the unexpected avidity of the rabbit TAL for metabolizing AA by a COX-independent pathway could be a function of CYP enzymes which in this tissue, were not primarily involved in drug metabolism but rather possessed the capacity to transform AA. The biological profile of these AA products of CYP enzymes was unknown as was their distribution in anatomical structures and tissues, a task that we undertook and one that has consumed the better part of two decades. This task was essential to identifying those renal structures that occupy essential roles in the regulation of ECFV in terms of their ability to generate eicosanoids. Although the highest levels/activity of CYP enzymes specific for AA metabolism had been localized to the renal outer medulla, exceeding those of the liver by more than three-fold, these findings could not assume functional significance until the anatomical structures that contain this activity were identified (6).
Our original study which uncovered the CYP pathway in the rabbit TAL, was conceived to define the ability of prostaglandins to affect the activity of Na\(^+\)-K\(^+\)-ATPase, the key enzyme in the nephron involved in sodium reabsorption, which is abundantly present in the TAL (3). Instead, this study identified AA metabolites that were generated by CYP enzymes present in a rabbit outer medullary cell suspension derived primarily from the TAL (ca 80%) (Fig. 1). Definition of the biological profile of these AA products was addressed in a follow-up study that had selected vasoactivity and the ability to affect the activity of Na\(^+\)-K\(^+\)-ATPase (the Na\(^+\) pump) as essential properties of putative regulators of renal function (4). It should be recalled that a hormone-inducible modulator of the Na\(^+\) pump had been long sought (designated the third factor, the natriuretic hormone) (7) and was considered to be an essential feature of the hypertensive state, namely, the exaggerated natriuresis produced by volume expansion in clinical and experimental hypertension had been ascribed to diminished activity of the Na\(^+\) pump in the medullary (m) TAL (8).

**CYP-AA metabolites**

Reverse phase-HPLC separation of CYP-derived AA products generated by the TAL revealed two peaks which differed in terms of their relative abilities to relax blood vessels *vis a vis* to inhibit Na\(^+\)-K\(^+\)-ATPase activity (9) (Fig. 2). Our
first attempt to define the principal arachidonate products in each peak was in error (4) and awaited completion of a collaborative study that identified definitively the structures of the major components of the AA products contained in the two peaks as separated by reverse phase-HPLC (9). The more polar peak (P2, Fig. 2) contained 20-COOH-AA which was devoid of vasoactivity but inhibited rabbit renomedullary Na⁺-K⁺-ATPase with a potency comparable to that of 20-hydroxyeicosatetraenoic acid (20-HETE) (IC₅₀ 3x10⁻⁷M). The less polar peak (P1, Fig. 2), contained two vasoactive components, 19- and 20-HETEs. However, 19-HETE did not affect the activity of purified renomedullary Na⁺-K⁺-ATPase whereas 20-HETE did. 20-HETE had biphasic effects on vascular rings, producing relaxation of rabbit precontracted mesenteric artery rings at 10⁻⁷M and contraction at 10⁻⁶M. These findings set the stage for examination of the functional consequences of altered production of 20-HETE, the principal CYP-AA metabolite synthesized by the TAL segment.

The Dahl salt-sensitive (SS) hypertensive rat demonstrates a primary lesion in Cl⁻ reabsorption, its elevation, in the TAL accounts for NaCl-induced hypertension (10). Deficient production of 20-HETE, the modulator of the Na⁺-K⁺-2Cl⁻ cotransporter, has been identified as the responsible factor that increases NaCl reabsorption with the production of hypertension (11). Repair of the deficiency in 20-HETE production by the TAL was effected by treatment with clofibrate to induce expression of ω hydroxylase via activation of peroxisome proliferator-activated receptor (PPAR) (12). Thus, pharmacological reversal of the deficiency in 20-HETE synthesis by the mTAL corrected the hypertension in Dahl SS rats. An additional effect of 20-HETE in the TAL segment that decreases NaCl reabsorption is the ability of 20-HETE to reduce the activity of the apical
intermediate conductance K⁺ channel (13). As the functional integrity of the cotransporter is dependent on available luminal K⁺ provided by K⁺ recycling via the K⁺ channel, 20-HETE-induced closure of the channel will diminish the activity of the cotransporter.

**TNFα, COX-2 and the TAL**

The discovery of the capacity of the TAL to synthesize tumor necrosis factor alpha (TNFα) (14) provided an impetus to examine the consequences of lipopolysaccharide (LPS) challenge on AA metabolism by the TAL (15). Prolonged exposure (ca 24 h) of cultured mTAL cells to LPS, an activator of cytokine production, resulted in expression of COX-dependent inhibition of ⁸⁶Rb uptake, mediated by PGE₂ in the mTAL that was activated by TNFα production in response to LPS challenge. This study demonstrated that a switching mechanism can be evoked in the TAL that displaced (replaced?) CYP-dependent AA metabolism with COX. Stokes had reported in 1979 that PGE₂ inhibited Cl-transport across the rabbit TAL (16).

We next addressed the ability of angiotensin II (ANG II) to affect ion transport in the mTAL as AT₁ receptors had been identified in this segment and ANG II was reported to inhibit the activity of the intermediate conductance K⁺ channel by stimulating production of 20-HETE in the mTAL (13,17). We hypothesized that a novel transport mechanism housed within the mTAL responds to ANG II via expression of a COX-dependent modulator of key transporters. Time and dose-dependent effects of ANG II on ion transport by the mTAL were discerned. Only high dose ANG II (10⁻⁶M), on short-term exposure (15 min), was dependent on a CYP product to inhibit ⁸⁶Rb uptake by mTAL tubules whereas on long-term exposure (> 3 h) to ANG II, a COX product, probably PGE₂, mediated the inhibitory effect on ⁸⁶Rb uptake of both high and low doses of ANG II. ANG II was also shown to increase TNF production by mTAL tubules, an effect that has been linked to COX expression. Long-term exposure to ANG II increased accumulation of TNFα mRNA associated with a five-to-six fold increase in production of TNFα. Further, the capacity of ANG II to stimulate PGE₂ production by mTAL tubules could be attenuated by TNFα antibodies. In a follow-up study, administration of anti-TNF antisera produced a further increase in blood pressure in rats receiving chronic ANG II infusion (18). We interpreted these findings to endorse an antipressor function for TNFα acting in concert with vasodilator-natriuretic prostanoids, principally PGE₂.

**Hypercalcemia, glucocorticoids and the TAL**

There are striking similarities between the effects of hypercalcemia on renal function and those induced by factors that either promote CYP-AA metabolism in the TAL or effect expression of COX-2 in this nephron segment. Namely, the inability to excrete a concentrated urine associated with the production of
natriuresis-chloruresis occur in response to hypercalcemia through stimulation of calcium-sensing receptors (CaR) found in several nephron segments including the mTAL (19,20). COX-2 mRNA and protein as well as PGE\(_2\) synthesis were increased in a dose- and time-dependent manner after exposure of primary cultures of mTAL cells to extracellular Ca (21). Thus, activation of CaR initiates an intracellular signaling mechanism that results in expression of COX-2 with production of PGE\(_2\), the eicosanoid responsible for the hypostenuria and natriuresis-chloruresis evoked by chronic hypercalcemia.

The most recent study on the regulation of oxygenases that metabolize AA was prompted by the findings that: 1) chronic dietary salt restriction produced a substantial increase in immunoreactive COX-2 in the TAL (22); and 2) COX-2 bears primary responsibility for morphogenesis of the nephron (23). The latter coincides with a sharp reduction in levels of glucocorticoid in the neonate. These observations served as the basis of a working hypothesis: adrenalectomy (ADX) will promote expression of COX-2 in the TAL segment of the nephron. ADX resulted in a well-defined pattern of COX-2 expression in the TAL that was initiated in the cortex and proceeded towards the medulla, thereby involving in excess of 30% of TAL cells (24). Sham-operated rats showed expression of COX-2 in less than 2% of TAL cells. Treatment of rats with glucocorticoids prevented recruitment of COX-2 positive cells.

**Conclusion**

These studies have established the essential role of eicosanoids in a key nephron segment. The CYP pathway of AA metabolism has been shown to be involved in regulating the activity of the cotransporter. Further, the expression of COX-2 in the TAL has been linked to activation of the renin-angiotensin system, glucocorticoid deficiency and hypercalcemia, all of which operate through a mechanism in which production of TNF\(\alpha\) by the TAL is pivotal. These studies should lead to the development of novel therapeutic strategies for the management of hypertension, diseases associated with sodium retention, and disorders of Ca metabolism (25).

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