Renal Arachidonic Acid Metabolism

The Third Pathway

MICHAL SCHWARTZMAN, MAIREAD A. CARROLL, NADER G. IBRAHAM,
NICHOLAS R. FERRERI, EMEL SONGU-MIZE, AND JOHN C. McGiff

SUMMARY Cells were isolated from the outer medulla of the rabbit kidney, primarily from the thick ascending limb of Henle’s loop (mTALH). These mTALH cells are heavily invested with a cytochrome P450-linked monooxygenase that represents the third pathway by which arachidonic acid is metabolized. After cell separation, approximately 80% of the cells proved to be mTALH in origin, based on electron microscopic criteria and immunofluorescent localization of Tamm-Horsfall protein, a specific marker for mTALH cells. The specific activity of alkaline phosphatase, a marker for proximal tubular cells, decreased threefold after separation of mTALH cells from outer medullary cells, associated with a fourfold increase in the capacity of the separated mTALH cells to metabolize arachidonic acid. Incubation of mTALH cells with [14C]-arachidonic acid resulted in formation of oxygenated metabolites, identified as two peaks (P1 and P2), which accounted for 30 to 40% of the recovered radioactivity. Formation of prostaglandin E2 and F2 accounts for only 3 to 5%. The chromatographic retention times of P1 and P2 were different from products of lipoxygenases. An inhibitor of cytochrome P450-dependent enzymes, SKF-525A (50 \mu M), reduced product formation by mTALH cells by more than 70%, while induction of cytochrome P450 increased product formation. Formation of P1 and P2 by cell-free homogenates of mTALH was wholly dependent on the presence of nicotinamide adenine dinucleotide phosphate, reduced form (NADPH), which suggests a NADPH-dependent cytochrome P450-linked monooxygenase pathway. Vasopressin and calcitonin (10^{-10} M to 10^{-7} M) stimulated release of arachidonic acid metabolites from mTALH cells. P2 yielded a principal product that proved to be a potent inhibitor of Na,K-ATPase, whereas the major component of P1 was a weak inhibitor. The latter material relaxed isolated blood vessels. In mTALH cells obtained from rabbits made hypertensive by aortic constriction, increased P1 and P2 formation could be demonstrated within 7 days. (Hypertension 7 [Suppl I]: I-136–I-144, 1985)

KEY WORDS • thick ascending limb of Henle’s loop • cytochrome P450-dependent monooxygenase • Na,K-ATPase • vasopressin • epoxides

The generation of prostaglandins and other oxygenated metabolites of arachidonic acid (AA) is a complex process initiated by the release of esterified AA from cellular lipids. Once liberated from membrane lipids by diverse stimuli (peptide hormones, neurotransmitters, and mechanical disruption) the free AA is rapidly metabolized. Metabolism of AA involves three enzymatic systems: cyclooxygenase, lipoxygenases, and cytochrome P450 mixed-function oxidases. The pathway by which AA is transformed depends on several factors, such as the tissue, the kind of stimulus, cofactor availability, and inhibitors. In the presence of oxygen, cyclooxygenase and lipoxygenase pathways will facilitate the sequence of hydrogen removal, double-bond rearrangement, and inclusion of an oxygen moiety to yield one of several unstable hydroperoxy fatty acids (HPETEs; Figure 1). The site of enzyme attack is critical in determining the final fate of AA, as the 11-HPETE formed by the action of cyclooxygenase is the precursor of all prostaglandins, while most other HPETEs (i.e., 5, 8, 12, and 15), produced by the action of lipoxygenases, may then form their corresponding monohydroxy fatty acids (HETEs). Alternatively, the 5-HPETE forms a labile
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Figure 1: Three major pathways of eicosanoid formation: cytochrome P450 monoxygenases, cyclooxygenase, and lipoygenases. The AA metabolites can be formed by oxygenation in the positions denoted, the examples of AA metabolites shown are 5,6 epoxide and 5,6 diol formed by monoxygenases and 5-HETE and leukotrienes formed by lipoygenases. Note that only the configuration of the cyclo-pentane ring is given for the classical prostaglandins PGD2, PGE2, and PGG2. TxA2 = thromboxane A2, TxB2 = thromboxane B2, MDA = malondialdehyde, LTC4 = leukotriene C4, LTD4 = leukotriene D4, LTE4 = leukotriene E4, LTB4 = leukotriene B4. HHT = (12S)-12-hydroxy-5,8,10 heptadecatrienoic acid, R-SH = glutathione, S-R = S-glutathionyl.

5-6 epoxide (LTA4) that is the precursor of the leukotrienes. The third system by which AA may be oxygenated in vivo is the cytochrome P450-dependent monoxygenase, a mixed-function oxidase system strictly dependent on molecular oxygen and nicotinamide adenine dinucleotide phosphate, reduced form (NADPH). This system can metabolize AA by three types of reactions (see Figure 1): (1) allylic oxidation leading to the formation of HETEs, (2) olefin epoxidation leading to the formation of four different epoxyeicosatrienoic acids (EETs) that can undergo hydrolysis by epoxide hydrolase to form the corresponding diol metabolites, the dihydroxyeicosatrienoic acids, (3) oxidation at ω and ω-1 positions to form the 20-HETEs and 19-HETEs respectively. As cytochrome P450 exists in multiple forms, the predominance of one of these reactions over others may be controlled by the isozyme composition of each tissue or cell type.

The pattern of AA metabolism in the kidney is distinct and presumably makes an important contribution to integrated renal function. Among the structures that can metabolize AA by the cyclooxygenase pathway to prostaglandins are the collecting tubules,1 the glomeruli,2 the medullary interstitium,3 and the blood vessels.4 Within the nephron, the convoluted tubules have low or negligible cyclooxygenase activity.4

Lipoxygenase pathways have been well characterized in leukocytes,5 platelets,6 and tumor cells.7 Jim et al8 have demonstrated lipoxygenase activity in rat glomeruli, glomerular epithelial cells, and homogenized cortical tubules. Winokur and Morrison9 described several lipoxygenase-derived products using subcellular fractions obtained from medullas of hydronephrotic and contralateral kidneys of rabbits. They did not associate products formed with a specific renal structure, however, and at least in hydronephrosis, products of lipoxygenase pathways may arise from blood elements (leukocytes) infiltrating renal tissues.9 Further, biochemical pathways and products that require intact cells for their demonstration may be overlooked.

The cytochrome P450 mixed-function oxidase system is comprised of three components: cytochrome P450 as the hemoprotein, a flavoprotein reductase identified as the NADPH-dependent cytochrome C reductase, and phosphatidylcholine, which serves to facilitate electron transfer in microsomal systems. Cytochrome P450, as the terminal oxidase of the drug metabolizing enzyme system, exists in multiple forms...
that differ in substrate specificity, positional specificity, and stereospecificity. Although the hepatic cytochrome P450 system is well characterized, less is known about renal cytochrome P450. The renal content of the components of the cytochrome P450-dependent mixed-function oxidase system has been measured and found to be smaller than that of the liver. The distribution of the components and activities of the mixed-function oxidase system have been studied in the rabbit kidney. The highest level of cytochrome P450 was present in the renal cortex. Similarly, renal monoxygenase activity was highest in the cortex. Only when the rabbits were treated with an inducer of cytochrome P450 activity such as 3-methylcholanthrene (3MC) was cytochrome P450 detected in the outer medulla.

Endou has described the distribution of the cytochrome P450 monooxygenase system (microsomal and mitochondrial) along the rabbit and rat nephron. Cytochrome P450 was localized primarily in proximal tubules with the highest concentration in the S3 segment, while nephron segments other than those of the proximal tubule did not possess cytochrome P450 as measured by the peroxidase assay. Using antibodies to four forms of hepatic cytochrome P450, Dees et al. demonstrated histochemically the existence of cytochrome P450 in rat and rabbit kidneys under conditions that induced enzyme activity. A study on the renal distribution of NADPH-dependent cytochrome C reductase, one of the components of the cytochrome P450 system, disclosed its presence in the cortex and outer medulla.

As mentioned before, cytochrome P450 is an inducible system and exists in multiple forms. Frequently, a particular form can be detected only after induction with a specific drug. Oliw et al. and Capdevila et al. have demonstrated the conversion of AA to several oxygenated metabolites by hepatic and renal cortical microsomes and by a reconstituted, purified, cytochrome P450 system in the presence of NADPH. Although the role of prostaglandins in the regulation of renal function has been well studied, the renal functional effects of AA metabolites arising from either lipoxygenases or cytochrome P450-dependent monoxygenases are virtually unknown. Initial studies suggest that products of AA arising from cytochrome P450-dependent enzymes exert actions on the vasculature and participate in the regulation of hormonal secretion. Jacobson et al. recently have reported that 5,6 EET, when injected into perfused rabbit cortical collecting tubules, was able to inhibit sodium transport.

The function of the nephron is segmented: as many as 12 segments have been identified that differ in morphological form, transport properties, and hormonal responsiveness. This arrangement has important functional implications when localizing a particular AA metabolite to a specific renal structure or nephron segment, a crucial first step in the definition of renal regulatory mechanisms mediated or modified by a product of AA metabolism. Moreover, the several pathways of AA metabolism are also localized segmentally within the nephron.

The nephron has been profiled in terms of distribution of prostaglandin-synthesizing capacity, based on immunohistofluorescence techniques for detecting cyclooxygenase. Of particular note was the reduced cyclooxygenase antigenicity in the thick ascending limb of Henle’s loop (TALH), which suggested low prostaglandin-forming capacity in this portion of the nephron. This region has been identified, at least in the medulla (mTALH), as a principal site of the inhibitory action of prostaglandin E2 (PGE2) on sodium chloride absorption. Our results suggest that the reduced amount of cyclooxygenase in the mTALH does not mean that the mTALH has a limited ability to metabolize AA as other pathways of arachidonate metabolism may predominate in a particular tissue. Further, the thin limb of Henle may be a source of PGE2.

Because of the importance of arachidonate metabolites to the regulation of renal function and the heterogeneous nature of the nephron with respect to ion transport and hormonal responsiveness, the pattern of AA metabolism should be related to specific cells within the nephron. The mTALH was of particular concern because of its pivotal role in the regulation of extracellular fluid volume, as well as containing the principal target cells for the most potent diuretic agents: furosemide, ethacrynic acid, and bumetanide. Moreover, the mTALH is involved in hypertension; Postnov et al. have observed decreased activity of Na,K-ATPase in the outer medulla of hypertensive rats and have suggested that a defect in the mTALH is responsible for the exaggerated natriuresis seen in human and experimental forms of hypertension.

We isolated cells from the mTALH to study AA metabolism, to identify and characterize the AA metabolites, and to determine the effects of hormones on formation of AA metabolites by mTALH cells. The use of cell isolates, obtained primarily from the medullary portion of the mTALH, was dictated by practical and theoretical considerations. Detailed biochemical analyses are possible as the yield of cells is relatively large, unlike that in microdissected tubules. We elected to use cell isolates initially rather than attempt to grow cells in culture because the latter frequently do not retain important properties of the parent cells. According to Thurman and Kaufman, “A major problem with hepatocytes in primary cell culture is that their cytochrome P450 declines rapidly, decreasing almost 80% during the first 24 hours in vitro.”

Separation, Characterization, and Identification of Cells from the Medullary Thick Ascending Limb of Henle’s Loop

We have developed a method for isolating a cell suspension obtained from the inner stripe of the outer medulla of the rabbit kidney and have studied these cells as described herein. After trypsin treatment, mechanical disruption, and separation by centrifugal elutriation, the resulting cell suspension consisted mainly
of mTALH cells. More than 90% of the cells were viable.

New Zealand white male rabbits (3.0–3.5 kg) were anesthetized with sodium pentobarbital (30 mg/kg), and the kidneys were flushed through their arteries with 0.9% saline. The inner stripe of the outer medulla was excised, cut into small pieces, and trypsinized. A single cell suspension was prepared by mechanical disruption, washed in phosphate buffered saline, and separated into several fractions by centrifugal elutriation. Assessment of eluted fractions by histochemical, biochemical, and morphological criteria showed that cells derived mainly from the mTALH were eluted at a flow rate of 19 ml/minute at 2000 RPM and 20°C. The resulting single cell suspension consisted mainly (80%) of mTALH cells; more than 90% were viable as determined by trypan blue exclusion, negligible release of lactic dehydrogenase into the medium, and electron microscopic appearance. The mTALH cells have been extensively characterized with the use of a variety of biochemical, histochemical, and immunological probes.

After separation by centrifugal elutriation, the cells obtained from the inner stripe of the outer medulla were highly enriched with mTALH cells based on the following biochemical and histochemical markers as well as morphological appearance. The specific activity of Na,K-ATPase, having the greatest activity in mTALH cells, is not a selective marker as it is found in all cells. This activity was increased more than twofold. Alkaline phosphatase, a marker of proximal tubule cells, was used as a negative marker of mTALH cells. It decreased by more than threefold when compared with the medullary cell suspension and more than ninefold when compared with renal cortical cells. Tamm-Horsfall protein is a specific marker for mTALH cells. After cell separation, the percent of cells showing immunofluorescent localization of Tamm-Horsfall protein agreed with the estimated percentage of mTALH cells of 80% based on electron microscopic criteria. The most direct evidence that the isolation and separation procedures yielded a cell population comprised primarily of mTALH cells was provided by electron microscopy. Based on electron microscopic criteria, approximately 80% were mTALH cells, which accords with a fivefold increase in specific activity of oxygenated AA metabolites produced by cells after separation from the medullary cell suspension.

We have also obtained evidence based on the effects of vasopressin stimulation of mTALH cells that the oxygenated products of AA did, indeed, arise from these cells. Thus, two segments of the nephron, the medullary collecting duct and the medullary ascending limb, exhibit responsiveness to vasopressin mediated by adenylyl cyclase–related mechanisms. We have also shown mTALH cell responsiveness to calcitonin, which was used by Eveloff et al. as a marker for mTALH cells. As vasopressin-sensitive receptors have been identified only in the medullary ascending limb and in medullary collecting ducts, and as collecting ducts represented less than 3% of the cells separated from the medullary cell suspension by centrifugal elutriation, these products must have arisen from mTALH cells. Finally, AA metabolites, not suppressed by meclofenamate, which possessed the chromatographic properties of the cytochrome P450-related AA metabolites, have also been observed by Schlondorff et al. in mTALH cells.

**Characterization of Arachidonic Acid Metabolites**

Incubation of mTALH cells (3 × 10⁶ cells/ml) with ¹⁴C-AA (7 μM) leads to rapid incorporation (within 30 minutes) of ¹⁴C-AA into cellular lipids, mainly phospholipids and triglycerides. During and following incorporation, radioactive products were released into the incubation medium and reached a maximal value at 60 minutes. Determination of the products released into the medium by radiochromatography and autoradiography of thin-layer chromatography plates showed a major peak consisting of two radioactive products migrating in the vicinity of 5-HETE standard (seen in autoradiograph of Figure 2 but not distinguishable on the radiochromatogram scan of Figure 3). The less polar peak for purpose of clarity is designated P₁ and the more polar peak, P₂ (Figure 2). Each peak has been shown to contain two to three products on gas chromatography.

Metabolic profiles before and after cell separation (TALH cells) were qualitatively similar; however, important quantitative differences were evident (Figure 3). These differences were expressed in terms of specific activity after thin-layer chromatography plates were cut and counted in a liquid scintillation counter.

![Figure 2. Autoradiography of ¹⁴C-AA oxygenated metabolites released from separated mTALH cells. Cells (3 × 10⁶/ml) were incubated with ¹⁴C-AA (7 μM) at 37°C in Krebs-Henseleit buffer (pH = 7.4). Aliquots (1 ml) were taken at indicated times, cells were removed by centrifugation, and the media extracted. The AA metabolites were separated by thin-layer chromatography 12-HETE = (12S)-12-hydroxy-5,8,10,14-eicosatetraenoic acid; 5-HETE = (5S)-5-hydroxy-6,8,11,14-eicosatetraenoic acid, PG = prostaglandin.](image-url)
The mTALH cells showed a fivefold increase in specific activity of $P_1 + P_2$ compared to the unseparated outer medullary cells: $4.37 \pm 2.07$ versus $0.80 \pm 0.44$ (mean $\pm$ sem) $\mu$g of AA converted to $P_1$ and $P_2$/mg of protein/30 minutes. No difference in specific activity was seen for cyclooxygenase products formed by outer medullary cells or mTALH cells. This finding suggests that these products were in fact formed by mTALH cells.

Further characterization of AA metabolites, related to identification of unknown products, was carried out by high-performance liquid chromatography (HPLC) together with on-line monitoring of radiolabeled AA metabolites and determination of the ultraviolet absorbance of the metabolites. Two major radioactive peaks were detected with normal-phase HPLC. Product 1 ($P_1$) had a slightly longer retention time than the authentic 8-HETE standard. Product 2 ($P_2$) had the same retention time as authentic 5-HETE standard. When the same samples were monitored for ultraviolet absorbance at 234 nm (wavelength maximum for conjugated dienes), ultraviolet absorbance was not detected. Similarly, absorbance was not seen at 276 nm (absorbance maximum for conjugated trienes). The sensitivity of detection by ultraviolet absorbance for the mono-HETEs was 2 to 5 ng. Given the amount of radiolabeled products ($\mu$g range), there was sufficient material for ultraviolet detection. The lack of ultraviolet wavelength at 234 nm or 276 nm suggested that the compounds lacked either a conjugated diene or triene structure and were not mono-HETEs or di-HETEs respectively. In view of these findings, reverse-phase HPLC was used to assist in further characterization of the unknown AA metabolites (Figure 4). The lack of ultraviolet absorbance at 234 nm or 276 nm was confirmed, and the radiolabeled metabolites ($P_1$ and $P_2$) were found to have retention times that differed from authentic HETE standards.

**Cytochrome P450 Activity and Arachidonic Acid Metabolism**

The possibility that these metabolites were derived from a cytochrome P450-dependent mechanism was further examined by measuring cytochrome P450 content and activity in different zones of the kidney, especially the inner stripe of the outer medulla — the source of the mTALH cells. The amount of cells obtained from each rabbit ($30-40 \times 10^6$) was not sufficient for cytochrome P450 assays. Cytochrome P450 content was determined spectrophotometrically in renal microsomes obtained from control rabbits and those treated with 3MC and $\beta$-naphthoflavone ($\beta$NF) to induce cytochrome P450 content. The results...
clearly indicate an increase of cytochrome P450 content in the inner stripe of the outer medulla (designated outer medulla in Table 1).

Depletion of cytochrome P450 content can be achieved by injecting cobalt. Cobalt is a known inducer of heme oxygenase activity, which increases heme degradation and thus depletes cytochrome P450, a heme-containing protein. When rabbits were treated with cobalt a marked decrease of cytochrome P450 content was noted in all zones of the kidney. Aryl hydrocarbon hydroxylase activity, a cytochrome P450-dependent enzyme, exhibited the same renal distribution pattern as cytochrome P450.

We also measured the effects of cytochrome P450 inhibitors (SKF-525A and metyrapone) and inducers (3MC and βNF) on metabolism of AA by mTALH cells. The mTALH cells were prepared from control rabbits and those treated with 3MC and βNF. We calculated the amount of radioactivity in AA metabolites (P1 and P2) as micrograms of AA converted to P1 + P2/mg of protein/30 minutes. A twofold increase in P1 and P2 formation occurred after 3MC and βNF treatment (2.45 and 4.29 µg of AA converted/mg of protein/30 min in cells from control and treated rabbits, respectively). Further, treatment of rabbits with CoCl2 caused a 50% decrease in the formation of P1 and P2. These changes in AA metabolism by mTALH cells correlated with changes in cytochrome P450 content in the zone from which the mTALH cells originated. When the amount of AA converted to these metabolites was calculated per nanomole of cytochrome P450, no significant change in the rate was observed among the different treatments, which indicates the high correlation between the two systems, metabolism of AA and cytochrome P450. In addition, SKF-525A, an inhibitor of cytochrome P450-mediated reactions through type I binding, inhibited P1 and P2 formation up to 90% at concentrations of 50 µM, while metyrapone, an inhibitor of cytochrome P450-mediated reaction through type II binding, at the same concentration (50 µM) did not inhibit product formation. Morrison and Pascoe used higher concentrations of metyrapone and demonstrated inhibition of ω-hydroxylation mediated by cytochrome P450 in renal microsomes. We were prevented from using these concentrations of metyrapone as it caused cell destruction. It is known that cytochrome P450 exists in multiple forms and differs with respect to substrate specificity, kinetic characteristics, and sensitivity to inducers and inhibitors. Our data suggest that the mTALH cell cytochrome P450 species that is sensitive to SKF-525A but relatively insensitive to metyrapone is responsible for AA metabolism in these cells.

We also localized the enzyme activity(ies) leading to the formation of P1 and P2 within the mTALH cells' membrane fraction. The renal cytochrome P450 system was localized to the membrane fractions, mitochondria, and microsomes; lipoxygenases are known to be associated with the cytosol. Conversion of 14C-AA to P1 and P2 by homogenates of mTALH cells was absolutely dependent on the addition of NADPH (Figure 5). Kinetic studies on AA conversion to P1 and P2 by homogenates indicated an apparent Km of 57 µM for AA and a Vmax of 29 nmol of AA converted/mg of protein/30 minutes.

**Hormonal Stimulation of Arachidonic Acid Metabolism in Cells from the Medullary Thick Ascending Limb of Henle's Loop**

As there is no significant pool of free AA inside the cells, 98–99% of AA being esterified to lipids, conversion of AA to oxygenated metabolites is limited by the availability of free AA. Several peptide hormones, in particular bradykinin and angiotensin II, are known to activate release of AA from cellular lipids and thereby stimulate the generation of oxygenated products. The mTALH cells were prelabeled with 14C-AA (0.4

### Table 1: Cytochrome P450 Content in Rabbit Kidney Under Control and Induced Conditions

<table>
<thead>
<tr>
<th>Renal zone*</th>
<th>Control</th>
<th>3MC and βNF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortex</td>
<td>0.138 ± 0.018</td>
<td>0.203 ± 0.017</td>
</tr>
<tr>
<td>Outer medulla</td>
<td>0.066 ± 0.006</td>
<td>0.120 ± 0.036</td>
</tr>
<tr>
<td>Inner medulla</td>
<td>0.026 ± 0.004</td>
<td>0.061 ± 0.004</td>
</tr>
</tbody>
</table>

Values are means ± SEM of eight animals

p < 0.05 when comparing control versus treatment group for each zone

3MC = 3-methylcholanthrene, βNF = β-naphthoflavone

*Renal microsomes from control and treated rabbits were assayed.
μCi/ml) for 60 to 90 minutes at 37°C. The cell suspension was washed, centrifuged, and resuspended in fresh buffer, then incubated for an additional 2 to 10 minutes with the following peptide hormones: bradykinin, angiotensin II, vasopressin, and salmon calcitonin (SCT). A dose-dependent stimulation (up to twofold) of radioactive AA metabolites (P1 and P2) was produced by vasopressin and SCT (10^-10 M to 10^-7 M). Bradykinin and angiotensin II, only at concentrations of 10^-6 M, affected product formation (approximately a 30% increase). The phosphodiesterase inhibitor 1-isobutyl-3-methylxanthine (10 μM) increased cyclic AMP (1 mM). Parathyroid hormone and isoproterenol, which do not affect adenylate cyclase in the mTALH segment,21 had no effect on AA metabolite formation by mTALH cells at concentrations of 10^-10 M to 10^-7 M.

After reverse-phase HPLC of AA metabolites formed by mTALH cells, as noted, two major peaks were detected using a radioactive flow detector (see Figure 4). Fractions containing P1 and P2 were collected separately, methylated, and subjected to further purification on normal-phase HPLC. The purified methylated products were further derivatized to the trimethylsilyl derivatives and subjected to analysis by gas chromatography and mass spectrometry, which revealed that each peak separated on HPLC contained more than one compound. A major compound associated with P1 can be defined as the methyl ester derivative of 5,6-epoxyeicosatrienoic acid. Unequivocal identification must be based on the characteristic ions of the 5,6-diol structure.

Biological Activities of Arachidonate Metabolites from Cells of the Medullary Thick Ascending Limb of Henle's Loop

When considering the possible biological importance of AA metabolites arising from a cytochrome P450-dependent pathway in mTALH cells, the capacity to inhibit Na,K-ATPase was judged to be of paramount importance.30 As AA and several of its oxygenated metabolites have been shown to inhibit Na,K-ATPase,30 stimulation of these products could be a link that expresses hormonal regulation of the transport function of the mTALH. For example, the natriuretic action of vasopressin36 and SCT37 may be mediated by an AA metabolite acting as an inhibitor of Na,K-ATPase.38 Generated in mTALH cells in response to stimulation of adenylate cyclase by the peptide hormone, P2 proved to be a potent inhibitor of Na,K-ATPase, whereas P1 was a weak inhibitor30 (Table 2). The purified Na,K-ATPase was prepared from canine heart38 and its activity assayed according to the method of Martin and Doty.43 However, P1, but not P2, induced relaxation of precontracted rabbit pulmonary artery rings. P1 (4 × 10^-8 M) caused 40% relaxation of the vascular rings while acetylcholine (5 × 10^-4 M) resulted in 36% relaxation. Higher concentrations of P1 (2.3 × 10^-7 M) and acetylcholine (10^-7 M) caused 40% relaxation, respectively. The effects of peptide hormones on product formation raise the important consideration of separate hormonal regulation of each of the major AA metabolites of mTALH cells (e.g., one of the products may participate in the control of salt transport while the other product may affect vascular resistance).

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Concentration (M)</th>
<th>% Inhibition of Na,K-ATPase</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>2.8 × 10^-7</td>
<td>9, 10</td>
</tr>
<tr>
<td>P2</td>
<td>2.0 × 10^-7</td>
<td>64, 65</td>
</tr>
<tr>
<td>Ouabain</td>
<td>5.0 × 10^-7</td>
<td>33, 35</td>
</tr>
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Values are means ± SEM

TABLE 2. Biological Activities of Arachidonic Acid Metabolites Formed by the Medullary Portion of the Thick Ascending Limb Cells of the Rabbit Kidney

Hypertensive Rabbits

As one of the products generated by mTALH cells is a potent Na,K-ATPase inhibitor, and as the activity of this enzyme is decreased in the outer medulla of hypertensive animals34 and a circulating ATPase inhibitor is found in hypertensive patients,44 we studied changes in AA metabolism in mTALH cells obtained from rabbits made hypertensive by suprarenal aortic coarctation. Rabbits (3.0-3.2 kg) were anesthetized, and the aorta, between the celiac and anterior mesenteric artery, was constricted by more than 75%. Sham-operated rabbits were used as controls. On the eighth postoperative day when blood pressure had stabilized, the mean arterial pressure under anesthesia ranged from 64 to 75 mm Hg for controls (n = 8) and 90 to 130 mm Hg for hypertensive rabbits (n = 8). The kidneys were flushed in situ with cold saline, and the inner stripe of the outer medulla was excised. After trypsinization and mechanical disruption of the tissue, two cell
fractions, mTALH cells and outer medullary cells depleted of mTALH cells, were isolated by centrifugal elutriation. 

The AA metabolism was determined by incubating 3 × 10^6 cells with 7 µM [3H]-AA for 30 minutes at 37°C. The radioactive products obtained after extraction were separated by thin-layer chromatography, and radioactivity counted after visualization with autoradiography. There was a twofold increase in P1, P2, and P3 formation by mTALH cells from rabbits made hypertensive compared with mTALH cells obtained from sham-operated rabbits (data are expressed as median and semiquartile values): 2.60 (2.2–4.66) versus 1.54 (1.24–2.34) µg of AA converted/mg of protein/30 minutes for hypertensive and sham-operated rabbits respectively (p < 0.05). In contrast, outer medullary cells depleted of mTALH cells produced less AA metabolites than mTALH cells; 0.55 (0.29–1.07) µg of AA converted/mg of protein/30 minutes. Further, there was little change between control and hypertensive rabbits in AA metabolites formed by outer medullary cells depleted of mTALH cells (p < 0.3). Therefore, in rabbits made hypertensive by constricting the aorta, metabolism of AA to oxygenated metabolites was selectively increased in mTALH cells. This finding suggests a role for these metabolites in hypertension (e.g., mediation of enhanced natriuresis to volume expansion in hypertensive animals).

The major finding of our studies is that the mTALH cells generate a naturally occurring ATPase inhibitor as a product of a cytochrome P450 pathway. This finding may provide answers (and future directions) to major questions raised by physiologists: “Particularly lacking is a clear understanding of the factors that control the activity of the enzyme in vivo and whether it participates in rapid adjustments of Na+ reabsorption or K+ secretion by the kidney tubule.” 44 Postnov and colleagues found that “... in chronic arterial hypertension there is a decrease in the activity of Na,K-ATPase, in the outer renal medulla, which suggests a reduction in the resorption capabilities of the ascending Henle’s loop with respect to sodium and water.” 45 We have found increased formation of AA metabolites by mTALH cells obtained from hypertensive rabbits. The presence of such an endogenous inhibitor has been speculated on; Fejes-Toth and Szendasi suggested that it “... seems plausible to assume that vasopressin inhibits active salt reabsorption in the thick ascending limb either directly or indirectly through the release of a humoral natriuretic substance of either renal or extrarenal origin.” 46 The interaction of vasopressin with mTALH cells has much broader implications, however, and could serve as a paradigm for circulating hormones and factors operating through a similar mechanism. The cytochrome P450-dependent pathway of AA metabolism in mTALH may act as a common mechanism mediating natriuresis in response to some peptide hormones as well as to natriuretic substance(s) including circulating inhibitors of Na,K-ATPase activity thought to be elevated in hypertension. 

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