Production and Actions of the Anandamide Metabolite Prostamide E2 in the Renal Medulla

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ABSTRACT

Medullipin has been proposed to be an antihypertensive lipid hormone released from the renal medulla in response to increased arterial pressure and renal medullary blood flow. Because anandamide (AEA) possesses characteristics of this purported hormone, the present study tested the hypothesis that AEA or one of its metabolites represents medullipin. AEA was demonstrated to be enriched in the kidney medulla compared with cortex. Western blotting and enzymatic analyses of renal cortical and medullary microsomes revealed opposite patterns of enrichment of two AEA-metabolizing enzymes, with fatty acid amide hydrolase higher in the renal cortex and cyclooxygenase-2 (COX-2) higher in the renal medulla. In COX-2 reactions with renal medullary microsomes, prostamide E2, the ethanolamide of prostaglandin E2, was the major product detected. Intramedullarily infused AEA dose-dependently increased urine volume and sodium and potassium excretion (15–60 nmol/kg/min) but had little effect on mean arterial pressure (MAP). The renal excretory effects of AEA were blocked by intravenous infusion of celecoxib (0.1 μg/kg/min), a selective COX-2 inhibitor, suggesting the involvement of a prostamide intermediate. Plasma kinetic analysis revealed longer elimination half-lives for AEA and prostamide E2 compared with prostaglandin E11. Intravenous prostamide E2 reduced MAP and increased renal blood flow (RBF), actions opposite to those of angiotensin II. Coinfusion of prostamide E2 inhibited angiotensin II effects on MAP and RBF. These results suggest that AEA and/or its prostamide metabolites in the renal medulla may represent medullipin and function as a regulator of body fluid and MAP.

Introduction

The concept that the kidney is the most important organ for long-term blood pressure control and renal dysfunction is a major cause of hypertension has gained increasing acceptance. Although the prohypertensive hormonal system of the kidney, i.e., the renin-angiotensin system, is well established, the kidney has also been proposed to contain an antihypertensive endocrine system (Muirhead, 1993). According to this hypothesis, the renal medulla responds to increased medullary blood flow by secreting a vasodepressor lipid termed medullipin. The antihypertensive effects of medullipin were proposed to be caused by three specific biologic properties: vasodilator, inhibitor of sympathetic activation, and promoter of renal salt and water excretion. Others have speculated on a possible relationship between medullipin and the mechanism of pressure natriuresis, whereby sodium and water excretion by the kidneys are increased in proportion to elevated renal perfusion pressure (Mattson, 2003). With respect to pressure natriuresis, it is known that glomerular filtration, total RBF, and cortical blood flow are finely regulated and do not change significantly in response to increased renal perfusion pressure, whereas medullary blood flow is poorly autoregulated and increases in proportion to elevated renal perfusion pressure. Pressure natriure-
sis is a local renal adaptive mechanism to increased arterial blood pressure. However, the identity of medullipin has remained elusive, but its elucidation undoubtedly has great potential for leading to new therapies for hypertension.

The present study tested the hypothesis that anandamide (AEA) and/or its metabolites represent medullipin. AEA is the N-acyl ethanolamide of arachidonic acid (AA). Best known as a major endocannabinoid in the brain (Devane et al., 1992), AEA has been noted for its high concentration in kidney (Koga et al., 1997; Long et al., 2011), although its distribution in this organ has not been established. Although roles for AEA have been identified for various physiological responses, such as antinociception (Cravatt et al., 2001), drug dependence (Schlosburg et al., 2009a; Ramesh et al., 2011), and inflammation (Schlosburg et al., 2009b), its function in the kidney is not well defined.

AEA has several characteristics consistent with the biological identity of medullipin. First, it is a neutral lipid and a derivative of arachidonic acid (Muirhead, 1993). After intravenous infusion into anesthetized rats, AEA elicited a prolonged vasodepressor response accompanied by bradycardia, consistent with inhibition of the baroreceptor reflex and sympathetic outflow (Varga et al., 1995). Indeed, the vasocontractant properties of AEA have been demonstrated on various arteriolar beds (Mair et al., 2010) including afferent and efferent arterioles of the kidney (Deutsch et al., 1997; Koura et al., 2004). Deutsch et al. (1997) showed that AEA inhibits the KCl-stimulated release of norepinephrine from sympathetic nerves on renal arterioles, consistent with its ability to inhibit sympathetic effector function. Finally, methanandamide, a methylated analog of AEA, increased urine volume (UV) and decreased mean arterial blood pressure after intrarenal medullary infusion in anesthetized rats, implying that medullary AEA can regulate body volume homeostasis and blood pressure (Li and Wang, 2006). Although some of the latter effects were blocked by known antagonists of AEA receptors, others, such as the effect of intramedullarily infused methanandamide on diuresis and blood pressure, were not, suggesting a cannabinoid receptor-independent mechanism.

The kidney is also unique in its high activities of the AEA-metabolizing enzymes fatty acid amide hydrolase (FAAH) and cyclooxygenase-2 (COX-2). FAAH is a membrane-bound amidase that catalyzes the hydrolysis of AEA to arachidonic acid and ethanolamine (Cravatt et al., 2001). FAAH is usually considered as an AEA-inactivating enzyme, because its products are inactive at cannabinoid receptors. However, arachidonic acid is itself an enzyme substrate and signaling molecule. COX-2, the “inducible” form of cyclooxygenase, is expressed constitutively in the kidney (Harris et al., 1994), particularly in the renal medulla (Yang, 2003). COX-2 was demonstrated to metabolize AEA to N-ethanolamide analogs of prostaglandins (Yu et al., 1997), termed prostamides. Whether prostamides have a functional role in the kidney or elsewhere in the body remains unknown. However, several prostamides, including prostamide E2, were detected in the kidneys of mice treated with AEA (Weber et al., 2004).

The present study addressed the relationship among AEA, COX-2, and FAAH in the kidney, with a particular focus on the difference between cortex and medulla. The renal excretory effects of AEA after its infusion into the renal medulla were studied in the absence and presence of a COX-2 inhibitor. Finally, the pharmacokinetic and cardiovascular properties of prostamide E2 after intravenous infusion were characterized. Our data highlight several additional properties of AEA or its COX-2 metabolite, prostamide E2, consistent with their identity as renomedullary neutral antihypertensive lipids.

Materials and Methods

AEA, prostamide E2, and prostamide F2α were purchased from Cayman Chemical (Ann Arbor, MI). Arachidonic acid was from Sigma (St. Louis, MO). The deuterated analogs, d₄-AEA, d₄-2-arachidonoylglycerol, and d₆-arachidonic acid, were from Cayman Chemical. All chemicals used in the study were of reagent grade or better.

Animals. Two- to 4-month-old male mice with a C57BL6 background were used in this study. The mice were generated by using a breeding colony of cannabinoid receptor and FAAH knockout mice established at Virginia Commonwealth University. The knockout strains have been maintained by backcrossing onto the C57BL6 background for approximately 12 generations. In some cases, male C57BL6/J mice purchased from The Jackson Laboratory (Bar Harbor, ME) were used. All experiments involving mice were conducted according to protocols approved previously by the Virginia Commonwealth University Institutional Animal Care and Use Committee.

Liquid Chromatography/Tandem Mass Spectrometry for Quantitation of Endocannabinoids and Fatty Acyl Amides. The endocannabinoids [AEA and 2-arachidonoyl glycerol (2-AG)] and the ethanolamides of the fatty acids (oleic acid and palmitic acid) were measured in renal cortex and medulla by LC-MS/MS as described previously (Ramesh et al., 2011). In brief, mice were euthanized, the kidneys were removed, and the renal cortex and medulla were rapidly dissected on ice, snap-frozen in liquid nitrogen, and stored at −80°C until the time of processing. On the day of processing, the preweighed tissues were homogenized in 1.4 ml of chloroform/methanol (2:1 v/v containing 0.2 M phenylmethylsulfonyl fluoride with 2 pmol d₄-AEA, 1 nmol d₄-2-arachidonoyl glycerol, 3.3 nmol d₄-palmitoyl ethanolamide, 3 nmol d₄-oleoyl ethanolamide, and 1 nmol d₆-arachidonic acid as internal standards). After adding ice-cold normal saline (0.5 ml), the samples were vortexed for 1 min, and the tubes were then centrifuged at 3200 g at 4°C for 10 min. The organic phase was separated, and the aqueous phase plus debris was extracted twice more with 0.8 ml of chloroform. The organic phases from the three extractions were pooled and dried under nitrogen gas, and the residue was reconstituted with 0.1 ml of chloroform and mixed with 1 ml of ice-cold acetone. After centrifuging at 1500g for 5 min at 4°C, the upper layer was collected and evaporated under nitrogen. Dried samples were reconstituted with 0.1 ml of methanol for analysis by LC-MS/MS in electrospray mode. The following ion transitions were monitored in positive mode, (348 → 62) and (348 → 91) for AEA; (356 → 62) for FAAH-d₄; (279 → 269) for 2-AG; (387 → 96) for 2-AG-d₄; (300 → 283) for palmitoyl ethanolamine (PEA); (304 → 62) for PEA-d₄; (326 → 62) and (326 → 309) for oleoyl ethanolamine (OEA); and (330 → 66) for OEA-d₄, and in negative mode: (303 → 259) and (303 → 59) for AA and (311 → 267) for AA-d₄. A calibration curve was constructed for each assay based on peak area ratios for the calibrators and internal standards. The extracted standard curves ranged from 0.03 to 40 pmol for AEA and 0.05 to 64 nmol for 2-arachidonoyl glycerol.

Immunohistochemical Analysis for COX-2 and FAAH in the Mouse Kidney. The kidneys were removed, cut longitudinally, and fixed in 10% neutral buffered formalin. The fixed tissue was embedded in paraffin, and 4-μm sections were cut. Immunostaining was performed as described previously (Li et al., 2007) by using a rabbit anti-COX-2 polyclonal antibody (1:50, Cell Signaling Technology, Danvers, MA) or a rabbit monoclonal anti-FAAH antibody (1:2000 dilution) and a goat anti-rabbit IgG secondary antibody (1:200, Jackson ImmunoResearch, West Grove, PA). The sections were incubated with the primary antibody (1:100 dilution) overnight at 4°C and then washed in PBS for 30 min. After washing, the sections were incubated with the secondary antibody for 1 h at room temperature. The sections were then washed in PBS for 30 min and stained with 100 μg/ml DAPI (4’,6-diamidino-2-phenylindole) stock solution (1:1 dilution) for 15 min. The sections were washed in PBS for 2 min and mounted using DPX mountant. The images were acquired with a Zeiss (Thornwood, NY) Axioscop microscope equipped with an AxioCam HR digital camera and AxioVision software (Carl Zeiss MicroImaging, Thornwood, NY). The immunohistochemical analysis was performed with the aid of NIH ImageJ software (Rasband, 2006).
Protocol 1: Intramedullary Interstitial Infusion of AEA and MAP

Surgical Preparation of Mice. Mice were anesthetized and devoid of normal control, normal goat or rabbit serum was used instead. The negative controls showed positive immunoreactivity.

Prostaglandine-Forming Activity in Kidney Microsomes with AEA as Substrate. Kidney cortical and medullary tissues from four to six mice were pooled and homogenized in ice-cold 0.25 M sucrose containing 25 mM Na-HEPES, pH 7.4, 1 mM EDTA, and Complete protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany). After centrifugation at 10,000 g for 10 min at 4°C, the supernatant was transferred and centrifuged at 100,000 g for 1 h. The microsomes were resuspended in the same buffer, aliquotted into several tubes, snap-frozen in liquid nitrogen, and stored at −80°C. The microsomal protein concentration was determined by the Bradford method using a commercially available kit (Bio-Rad Laboratories, Hercules, CA).

Reactions (100 μl) contained 1 mg/ml microsomal protein, 0.1 M Tris-HCl, pH 8.0, 1 mM epinephrine, 1 mM glutathione, and 0.1 M AEA. The reactions were incubated at 37°C for 30 min and stopped by the addition of 15 μl of 2 N HCl followed by 300 μl of 2:1 (v/v) chloroform/methanol. The samples were vortexed for 90 s and centrifuged for 3 min. The bottom layer was transferred to a glass conical tube, and AEA-d8 (5 ng) was added as external standard. The samples were dried under nitrogen gas and reconstituted in methanol for analysis by LC-MS/MS as described previously (Weber et al., 2004) with some modification. The flow rate was 0.2 ml/min. The column was a Luna C8 3 μm (100 × 2.0 mm) column (Phenomenex, Torrance, CA) maintained at 40°C temperature. The entire liquid chromatography eluent was directed into the electrospray ionization source. The mass spectrometer was switched between positive ion mode (for AEA, prostamides, and d8-AEA) and negative ion mode (for arachidonic acid). The ion transitions monitored were: (396 > 378) and (396 > 62) for prostamide E2; (356 > 62) for AEA-d8; (398 > 380) and (398 > 62) for prostamide F2α; and (303 > 259) for arachidonic acid. Calibration curves were constructed for prostamide E2 and arachidonic acid.

Western Blot Analysis. Western blot analysis was performed as described previously (Zou et al., 2001). In brief, kidney cortical or medullary microsomal samples (20 μg) were electrophoresed through a SDS-polyacrylamide gel electrophoresis gel together with a protein size standard followed by electrophoretically onto a nitrocellulose membrane. The membrane was probed for FAAH or COX-2 protein by using the anti-FAAH or anti-COX-2 antibodies described above at 1:2000 and 1:50 dilutions, respectively. After washing, the membranes were incubated for 1 h with 1:3000 horseradish peroxidase-labeled secondary antibody. Images were developed by using enhanced chemiluminescence detection solution (Thermo Fisher Scientific, Waltham, MA) followed by exposure to Kodak X-Omat Blue film (Eastman Kodak, Rochester, NY). The intensities of the protein bands on the image were quantified by using ImageJ analysis software (National Institutes of Health, Bethesda, MD). To normalize for small differences in protein loading, the blots were stripped and reanlyzed with an anti-β-actin primary antibody (1:2000 dilution; Sigma).

Surgical Preparation of Mice. Mice were anesthetized and prepared surgically for each of the following protocols as described previously (Li et al., 2005). In brief, after anesthesia with ketamine (Ketaject, Phoenix Pharmaceutical, Inc., St. Charles, MO; 30 mg/kg i.p.) and thiobutabarbital (Inactin, Sigma; 50 mg/kg i.p.), the mice were placed on a thermostatically controlled warming table to maintain body temperature at 37°C. After tracheotomy, catheters were placed in the jugular vein and carotid artery for intravenous infusions and measurements of MAP, respectively. For experiments where RBF was measured, a flow probe (2 mm; Transonic Systems Inc., Ithaca, NY) was placed around the left renal artery, and RBF was measured as described previously.

Protocol 1: Intramedullary Interstitial Infusion of AEA and Measurement of MAP, Urinary Formation, and RBF. The technique for construction and placement of a catheter for renal medul-
Results

Levels of AEA and 2-Arachidonoyl Glycerol in Mouse Renal Cortex and Medulla. To assess the distribution of AEA in the kidney, the cortex and medulla from kidneys of C57BL6 mice were dissected and analyzed for endocannabinoids and other fatty acyl ethanolamides (Fig. 1). The AEA level was 2.4-fold higher in the renal medulla compared with the cortex (26.0 versus 10.7 pmol/g; \( p < 0.05 \)). Two other fatty acyl ethanolamides, oleoyl ethanolamide and palmitoyl ethanolamide, were also higher in the renal medulla than in cortex (3.3- and 4.6-fold, respectively; \( p < 0.05 \)). In contrast, 2-arachidonoyl glycerol levels did not differ between the renal cortex and medulla (11.4 and 10.4 nmol/g, respectively).

Distribution of COX-2 and FAAH in the Renal Cortex and Medulla. The distributions of the COX-2 and FAAH proteins in microsomes prepared from the kidney were studied by Western blot analysis and immunohistochemical localization (Fig. 2). In the Western analysis (Fig. 2A), immunoreactive bands consistent with FAAH (61 kDa) and COX-2 (74 kDa) were detected in the kidney microsomes. The two proteins showed opposite abundance profiles in the renal cortex compared with medulla with the FAAH immunoreactive band more intense in the cortex microsomes and the COX-2 band more intense in medulla microsomes. Quantification using \( \beta \)-actin as a normalizing control indicated 3.6-fold higher FAAH protein in the cortex compared with medulla and 6.1-fold higher COX-2 in the medulla compared with cortex (Fig. 2B; \( p < 0.05 \)). The results of immunohistochemical analyses of FAAH and COX-2 were in good agreement with the Western blot findings (Fig. 2C). FAAH immunostaining was most intense in the cortex region, with both proximal and distal tubule cells showing positive staining, in contrast to the weak or absent staining of cells in glomerular structures. In the medulla, FAAH staining was more selective, seeming to be limited to collecting ducts. The staining pattern with COX-2 in the cortex compared with the medulla was distinct from that of FAAH (Fig. 2C, bottom). In the renal cortex, positive COX-2 staining was limited to scattered cells or groups of cells in thick ascending limb tubules, particularly in regions where these cells came into contact with or were in proximity to glomeruli. This staining pattern is consistent with the reported expression of COX-2 in cells of the macula densa. In the medulla, COX-2-positive staining was detected in tubular segments with weak or otherwise minimal staining of collecting ducts.

Hydrolysis and Prostaglandin E2 Formation from AEA by Cortical and Medullary Microsomes. Figure 3 presents a comparison of the rates of AEA hydrolysis to arachidonic acid and oxidation to prostaglandin E2 by cortical and medullary microsomes with representative chromatograms. The kidney cortical microsomes showed a significantly higher rate of AEA-dependent arachidonic acid formation compared...
with medullary microsomes (928 ± 98 versus 787 ± 79 pmol/mg/h, respectively) (Fig. 3A), consistent with the cortex having higher FAAH activity. In contrast, the renal medulla microsomes were more active than cortical ones in forming prostamide E2 (33.4 ± 7.5 versus 20.4 ± 3.8 pmol/mg/h, respectively; p < 0.05) (Fig. 3B). Prostamide E2 was not detected in reactions containing only microsomes or only reaction components (data not shown).

Effect of Medullary Infusion of AEA on Blood Pressure, Urine Volume, and Sodium Excretion and Its Inhibition by a Selective COX-2 Inhibitor. An acute renal function study was performed by using anesthetized mice with surgically implanted catheters for measuring renal perfusion pressure and urine formation. These results are presented in Fig. 4. After a 1.5-h equilibration period and two 15-min control periods (labeled C1 and C2 in Fig. 4), addition of AEA to the medullary infusion solution was tested. Medullary infusion of AEA had little apparent effect on blood pressure, but increased urine flow and sodium and potassium excretion adjusted for kidney weight. The effects of AEA were dose-dependent between 15 and 60 nmol/kg/min. Removal of the AEA from the medullary infusion solution resulted in the urine flow and sodium excretion values returning toward the control level. Pretreatment of the mice with an intravenous infusion of celecoxib, a COX-2-selective inhibitor, at a rate of 0.1 g/kg/min for 30 min completely blocked the effects of AEA on both urine flow and excretion of sodium and potassium. This observation suggests that COX-2 metabolites of AEA mediate the diuretic and natriuretic effects of AEA during its intramedullary infusion in the mouse kidney.

Kinetics of AEA and Prostamide E2 after Intravenous Bolus Injection. Plasma samples from control C57BL6 mice before and after intravenous bolus doses of AEA or prostamide E were analyzed for AEA and prostamide E2 levels (Fig. 5). Basal levels of AEA (3–8 nM) but not prostamide E2 (data not shown) were detectable in plasma collected immediately before the bolus intravenous injection (3–8 nM; 1–3 ng/ml). After an intravenous AEA bolus (3.6 mg/kg; Fig. 5A), AEA exhibited a peak plasma level by 5 min after administration (489 ng/ml), which declined subsequently with a half-life of 20 to 30 min. No prostamide E2 was detected in the plasma of the AEA-injected mice. Intravenous injection of a prostamide E2 bolus resulted in a maximal plasma concentration of prostamide E2 by 5 min after injection (141 ng/ml; Fig. 5B). There was no apparent change in plasma AEA concentration after prostamide E2 dosing, which was consistent with the COX-2 metabolite being resistant to FAAH-mediated hydrolysis (J. K. Ritter and P.-L. Li, unpublished data). Prostamide E2 exhibited an elimination half-life of 21 min. An unidentified prostamide E2 metabolite
having identical parent ion ([MH]+ 396) and product ion ([MH]+ 378) as prostamide E2 was detected in the prosta-
mide E2 multiple reaction monitoring chromatograms. The
signal for this metabolite was detected only in mice injected
with prostamide E2 and became more intense than prostama-
die E2 by 30 min and later times after injection, suggesting
higher abundance. The elimination of this unknown isomeric
metabolite paralleled that of prostamide E2 (half-life of 26
min).

**Effect of Intravenous Prostamide E2 Infusion on Blood Pressure and RBF.** The influence of intravenously
infused prostamide E2 on MAP and RBF was investigated in
anesthetized mice. Representative blood pressure and RBF
tracings from a single animal showing the effects of prosta-
mide E2 on these parameters are shown in Fig. 6A. Prosta-
mide E2 had modest, but statistically significant, effects on
MAP and RBF under control conditions. At the highest dose
tested (120 nmol/kg/min) prostamide E2 decreased MAP by
7% from 99.4 to 92.6 mm Hg and increased RBF by 23% (4.7
to 5.8 ml/min/g kidney weight) (Fig. 6B).

**Inhibition of Ang II-Mediated Increases in MAP and
Reductions of RBF by Prostamide E2.** The influence of
prostamide E2 was also evaluated on Ang II-induced eleva-
tion in MAP and reduction of RBF. Administration of the Ang
II bolus transiently elevated MAP and decreased RBF, but
both of these effects were inhibited by prostamide E2 (see
Fig. 7A for representative tracings). The summarized data in
Fig. 7B show that whereas the Ang II bolus alone elevated
MAP by an average 22 mm Hg, this response was reduced
46% to 12 mm Hg by prostamide E2 (Fig. 7B, left). Likewise,
Ang II reduced RBF by 48% (4.6 to 2.4 ml/min/g kidney
weight) in the presence of vehicle and by 23% (3.3 to 4.2
ml/min/g kidney weight) in the presence of infused prosta-
mide E2 (Fig. 7B, right). Similar antagonistic effects of prosta-
mide E2 were observed when Ang II was administered by
continuous intravenous infusion. In tracings from a repre-
sentative animal (Fig. 7C), infusion of Ang II at increasing
concentrations (5, 10, and 20 ng/kg/min) elevated MAP and
decreased RBF in a concentration-dependent manner (Fig.
7C, left), and these effects were inhibited by the presence of
prostamide E2 (Fig. 7C, right). Summarized data showing
significant inhibition of Ang II effects at the intermediate
and high Ang II concentrations are shown in Fig. 7D.

**Discussion**

The purpose of this study was to evaluate AEA and its
prostamide metabolites for their possible relationship to the
renomedullary neutral antihypertensive lipid medullipin
(Muirhead, 1993). The observation that AEA is enriched in
the mouse renal medulla relative to cortex (Fig. 1A) has not
been described previously. The AEA enrichment in the renal
medulla is not clearly understood but may be caused by
relatively low levels of FAAH in medullary cells. The pre-

cence of AEA in neurons is well known, but it is also present
in cells that are non-neuronal in origin (Liu et al., 2006). In
the kidney, AEA has been identified in cultured renal micro-
vascular endothelial cells and glomerular mesangial cells
(Deutsch et al., 1997). Based on the hypothesis that AEA or
an AEA metabolite represents medullipin, it may be specu-
lated that AEA is present in the lipid-rich granules of re-

omedullary interstitial cells, the proposed source of medul-

lipin, but further studies are needed to confirm this
possibility.

It was also of interest to evaluate the distribution in the
kidney of two known AEA-metabolizing enzymes, FAAH and
COX-2, in relation to that of AEA. Multiple lines of evidence
support opposing patterns of FAAH and COX-2 expression in
the renal cortex and medulla. FAAH protein (Fig. 2) and a
measure of its activity, hydrolysis of AEA to arachidonic acid
(Fig. 3), were higher in the cortex than medulla, whereas the
pattern for COX-2 was the opposite. Although the kidney has
been reported to have high FAAH (Long et al., 2011), its
localization in the kidney has never been studied to our
knowledge. FAAH immunostaining was strong in various
cortical tubular segments including proximal convoluted tu-
bule, thick ascending limbs of the Loop of Henle, and cortical
distal and connecting tubules. Some positive staining of
FAAH was evident in glomeruli, but the intensity was lower.
than that in tubules. These immunohistochemical-based observations agree with the higher FAAH levels observed by Western blotting and enzymatic analysis. Increased FAAH activity in the renal cortex could at least in part explain the relatively low levels of AEA there and suggests the physiologic importance for maintaining low AEA concentrations in the renal cortex. Alternatively, AEA in conjunction with high FAAH in the renal cortex could serve as a source of arachidonic acid for prostaglandin synthesis in the tubuloglomerular feedback mechanism (Araujo and Welch, 2009). In the renal medulla, the inner medullary collecting ducts exhibited intense positive FAAH immunostaining. Further research is necessary to understand the role of FAAH and the regulation of AEA synthesis and release in the cortical and medullary regions of kidney.

In contrast, the COX-2 protein and an associated enzymatic activity (conversion of AEA to prostamide E2) both were higher in the renal medulla compared with the cortex (Figs. 2 and 3). This differential expression of COX-2 in the renal medulla was supported by 

**Fig. 5.** Plasma clearance of AEA and prostamide E2. Intravenous bolus doses of AEA or prostamide E2 were administered into mice, and plasma samples (50 μl) were then collected in EDTA tubes at timed postdose intervals and analyzed for the indicated lipids by LC-MS/MS. A, representative chromatograms for prostamide E2 monitored by using the 396378 multiple reaction monitoring show prostamide E2 (in black) with accompanying formation of an unidentified isomeric metabolite, which elutes slightly later (6.14 min) than prostamide E2 (5.51 min). B, representative chromatograms for AEA. C, plasma prostamide E2 levels after a prostamide E2 bolus (3.6 μmol/kg body weight). D, plasma AEA concentrations after an AEA bolus (3.6 μmol/kg body weight). Values represent the mean ± S.E.M of the lipid concentrations in units of ng/mL. (n = 4 per group).

**Fig. 6.** Effect of prostamide E2 infusion on mean arterial pressure and renal blood flow. A, representative tracings from a mouse given prostamide E2 by continuous intravenous infusion are shown. Effects on MAP (black tracing) and RBF (gray tracing) are shown. The prostamide E2 infusion rate was 30, 60, or 120 nmol/kg/min. B, summarized data for the average effect of prostamide E2 for each of the two 10-min treatment periods at each infusion rate. *, a significant difference versus control group (p < 0.05) (n = 7.)
In the acute renal function studies, a link between AEA and COX-2 is suggested by the data (Fig. 4). When AEA was infused into the renal medulla, urine flow and sodium excretion were increased. However, this effect was completely prevented if the mice were pretreated with a selective COX-2 inhibitor. This finding indicates that a COX-2 metabolite of AEA indirectly mediates the renal excretory effects of AEA. Using a similar experimental approach in rats, Li and Wang (2006) showed that the intramedullary infusion of the methyl analog of AEA, methanandamide, stimulated urine volume and reduced MAP (Li and Wang, 2006). Methanandamide has similar cannabinoid receptor-stimulating specificity to AEA but is resistant to FAAH-mediated hydrolysis (Abadji et al., 1994). The effect of intramedullarily infused methanandamide was not blocked by pretreatment with either a cannabinoid receptor-1 or vanilloid receptor antagonist, indicating a unique mechanism. It is plausible that this cannabinoid-independent mechanism for methanandamide also involves

Fig. 7. Angiotensin II intravenous dose response of mean arterial pressure and renal blood flow with and without prostamide E2 pretreatment in mice. A, representative tracings show the effect of an intravenous bolus injection of Ang II (50 ng/kg) and Ang II given after prostamide E2 pretreatment (120 nmol/kg/min) on MAP (black lines) and RBF (gray lines). B, summarized data for acute bolus intravenous injection of Ang II in the absence and presence of prostamide E2 on MAP and RBF. The data are presented as the mean ± S.E.M. expressed as the percentage of change from the basal level. C, representative tracings show the dose response of a continuous intravenous infusion of Ang II (5–20 ng/kg/min) and the effect of prostamide E2. D, summarized data for the effect of continuous intravenous infusion of Ang II with or without prostamide E2 (n = 7). *, p < 0.05; **, p < 0.01, versus control group.
In summary, the results of the present study demonstrate that AEA or one of its COX-2 metabolites, prostamide E2, shares several properties in common with those proposed for medullipin, the antihypertensive lipid with vasodilator, sympatholytic, and diuretic/natriuretic actions. In addition to its neutral lipid character, its close relationship to arachidonic acid, and an apparent role for metabolism in mediating its antihypertensive effects, AEA infused in the renal medulla is diuretic and natriuretic, and this effect is mediated by COX-2. Thus, we propose that AEA or prostamide E2 may represent medullipin. A key to this hypothesis is that AEA or its COX-2 metabolites are synthesized in the renal medulla and released in response to increases in renal medullary blood flow. Studies in our laboratory are ongoing to address this key issue.

**Authorship Contributions**

**Participated in research design:** Ritter, C. Li, Xia, Poklis, Lichtman, Dewey, and P.-L. Li.

**Conducted experiments:** Ritter, C. Li, Xia, Poklis, and Abdullah.

**Performed data analysis:** Ritter, C. Li, Xia, Poklis, Lichtman, Abdulla, Dewey, and P.-L. Li.

**Wrote or contributed to the writing of the manuscript:** Ritter, C. Li, Xia, Poklis, Lichtman, Abdullah, Dewey, and P.-L. Li.

**References**


Abdullah, Dewey, and P-L. Li.


