

Cytochrome P450 Metabolites of Arachidonic Acid Are Potent Inhibitors of Vasopressin Action on Rabbit Cortical Collecting Duct

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Abstract

AA is metabolized by a cytochrome P450, NADPH-dependent epoxidase to four regioisomeric epoxyeicosatrienoic acids (EETs). The EETs are further hydrated enzymatically to their respective diols, *vic*-dihydroxyeicosatrienoic acids (DHETs). We studied the effect of pretreatment with DHETs on $10 \mu\text{U}/\text{cm}^2$ arginine vasopressin (AVP)-stimulated hydraulic conductivity (L_p) ($L_p \times 10^{-7} \text{ cm/atm/s}$, mean \pm SE) in rabbit cortical collecting ducts (CCDs) perfused in vitro at 37°C . At 10^{-6} M all four DHETs were potent inhibitors of the hydroosmotic effect of AVP. 14,15-DHET was the most potent isomer; it reduced AVP-induced L_p from a control value of 234.75 ± 11.7 , $n = 17$, to a value of 95.2 ± 8.39 , $n = 5$, $P < 0.0001$, a reduction of AVP-mediated water flow of 60%. The inhibitory effect of 14,15-DHET was dose dependent and significant to nanomolar concentrations. 14,15-DHET at 10^{-7} M was as potent an inhibitor of AVP's activity as was 10^{-7} M PGE₂. AVP's hydroosmotic effect is mediated through its intracellular second messenger, cAMP. 8-*p*-Chlorophenylthio-cAMP (CcAMP) at 10^{-4} M induced a peak L_p of 189.6 ± 11.0 , $n = 8$; pretreatment with 10^{-6} M 14,15-DHET reduced CcAMP-peak L_p to 132.0 ± 13.4 , $n = 5$, $P < 0.01$, demonstrating a post-cAMP effect. Gas chromatography/mass spectroscopy suggests that EETs are present in extracts purified from CCDs. We conclude that cytochrome P450 epoxidase eicosanoids are potent inhibitors of the hydroosmotic effect of vasopressin and are endogenous constituents of normal CCDs, the major target tissue for AVP.

Introduction

Systematic study of the oxygenated metabolism of AA in health and disease has documented the importance of its metabolites to renal physiology and pathophysiology. Eicosanoids have been implicated in the renal response to peptide hormones and other cellular messengers. They affect renal circulation, contribute to inflammatory processes within the kid-

ney, and have important effects on solute and water transport (1–4).

AA can be metabolized by three distinct enzymatic pathways: (a) by cyclooxygenase to form biologically active PGs and TXs; (b) by lipoxygenases to form hydroperoxy fatty acids, monohydroxy fatty acids, and leukotrienes; and (c) by a recently described cytochrome P450 NADPH-dependent epoxidase (5–10). This third pathway of AA metabolism has been documented in the kidney (5, 7, 8). Epoxidation of each of the double bonds of AA generates four regioisomeric epoxides: the 5,6-, 8,9-, 11,12-, and 14,15-epoxyeicosatrienoic acids (EETs).¹ The epoxides can be hydrated by cytosolic epoxide hydratases to form the corresponding diols: 5,6-, 8,9-, 11,12-, and 14-15-dihydroxyeicosatrienoic acids (DHETs) (Fig. 1).

The presence of a cytochrome P450 NADPH-dependent monooxygenase in numerous tissues including the kidney has been documented and potentially important biologic actions of P450 eicosanoids in several physiologic systems have been observed (10–18). 5,6-EET has been shown to suppress sodium absorption and potassium secretion in microperfused rabbit cortical collecting ducts (CCDs) (11). In a cell preparation from medullary thick ascending limb of Henle, the production of metabolites of this pathway is altered by drugs, hormones, and maneuvers that result in experimental hypertension (16). NaK ATPase activity in thick-limb cells is reduced by these products (12, 16). Finally, recent studies on toad urinary bladders have shown that EETs and DHETs inhibit vasopressin-induced osmotic water flow (18).

We report here studies that examined the effect of diols (DHETs) of renal P450 epoxidase on the hydroosmotic response of the rabbit CCD to arginine vasopressin (AVP) and the cell permeant, phosphodiesterase-resistant cAMP analogue, 8-*p*-chlorophenylthio-cAMP (CcAMP).

Methods

Experiments were performed using the technique of in vitro microperfusion of isolated nephron segments as originally described by Burg et al. (19). Female New Zealand white rabbits were maintained on ad lib tap water and chow. They were killed by decapitation and the left kidney was rapidly exposed and removed. 1-mm-thick slices of kidney were made and these were placed in ice-chilled medium that had the same composition as the bath solution described below. A slice was transferred in the same chilled medium to a dissecting microscope where individual CCDs were dissected freehand with sharpened forceps. An isolated CCD (1–2.5 mm long) was then transferred to a thermostatically controlled lucite bath chamber on an inverted micro-

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1. Abbreviations used in this paper: CcAMP, 8-*p*-chlorophenylthio-cAMP; CCD, cortical collecting duct; DHET, dihydroxyeicosatrienoic acid; EET, epoxyeicosatrienoic acid; L_p , hydraulic conductivity; m/z , mass/ionic charge ratio; NICI GC/MS, negative ion chemical ion gas chromatography/mass spectroscopy; PFB, pentafluorobenzyl; R_t , retention time.

appropriate dilutions were made to obtain stock solutions such that the volume of ethanol vehicle added to the bath was the same for all experiments. Bath exchanges and collections for control and experimental conditions were performed according to the time course and methods described in the previous protocol.

Comparison of 14,15-DHET vs. PGE₂ on the AVP response. In these experiments we compared the effect of pretreatment with equimolar concentrations (10^{-7} M) of 14,15-DHET and PGE₂ on the response of CCDs to $10 \mu\text{U}/\text{cm}^2$ AVP. Other than the concentrations of each arachidonate metabolite that was present in the bath, the protocols followed were the same as above.

Effect of 14,15-DHET on CcAMP response. As initial studies to determine the mechanism by which DHETs were inhibiting the AVP response, we also examined whether 14,15-DHET would affect the hydroosmotic response of CCDs to the intracellular second messenger of vasopressin, cAMP. Tubules were perfused and bathed as described, then pretreated with either vehicle or 10^{-6} M 14,15-DHET, and basal collections were made for J_v and L_p . Subsequently, the bath was exchanged to one containing vehicle or DHET and 10^{-4} M CcAMP and peak response was measured. In CcAMP experiments the peak response was defined as above and occurred within 20 to 50 min after exposure to CcAMP.

Analysis of harvested CCDs for epoxigenase metabolites (Fig. 2). Rabbit CCDs were freehand dissected from collagenase perfused kidney slices in ice-chilled dissection medium using a dissection microscope as described above. Care was taken to remove adhering thick limbs from the individual CCDs. The lengths of the tubules were measured by eyepiece reticle. Dissection was completed within 60 min after death. Harvested tubules were transferred with minimal dissection medium to glass test tubes containing ice-chilled HPLC reagent grade methanol.

The CCDs were homogenized in ice-cold 0.2 M KCl containing 40% CH₃OH and 0.01% BHT (wt/vol). After adding 0.02 μCi of [5,6,8,9,11,12,14,15-³H]14,15-EET (150–170 Ci/mmol) in ethanol as recovery standard, an equal volume of cold CHCl₃ was added with vigorous mixing. After phase separation the aqueous layer was extracted with 2 vol of CHCl₃-CH₃OH (2:1).

The combined organic extracts were purified as described (24). Briefly, the lipid extract was hydrolyzed in the presence of 0.2 M KOH, 80% CH₃OH for 60 min at 40°C under an argon blanket. After neutralization and extraction into ethyl acetate the samples were purified by reverse-phase HPLC as described (25). HPLC fractions with retention times corresponding to those of synthetic EETs were collected, evapo-

rated under argon, and derivatized to the corresponding pentafluorobenzyl (PFB) derivatives. The EET-PFB esters were purified by reverse-phase HPLC as described (25). Samples were analyzed by NICI GC/MS on a Nermag quadrupole instrument (model R1010C) interfaced to a gas chromatograph (Varian Associates, Palo Alto, CA) using a 30-m SPB5 fused silica capillary column (0.32 mm i.d., 0.25- μm coating thickness; Supelco, Inc., Bellefonte, PA) with helium and methane as carrier and reagent gases, respectively. Instrumental conditions were as follows: analyzer pressure, 6.4×10^{-6} Torr; source temperature, 200°C; electron energy, 90 eV; dynode, +5 kV. For analysis, the GC column temperature was programmed from 100 to 310°C at 20°C/min and held at 310°C for 10 min. Injections were made in hexane.

Reagents

AA was from NuChek Prep, Elysian, MN. Radiolabeled AA was from Amersham Corp., Arlington Heights, IL. AVP, PGE₂, and CcAMP were purchased from Sigma Chemical Co., St. Louis, MO. All solvents were reagent grade. Radioactive tritium-labeled inulin was purchased from DuPont NEN Products Co., Boston, MA.

Statistics

Data are presented as mean \pm SE; statistical comparisons were made using one-way analysis of variance. A difference of $P < 0.05$ was considered statistically significant.

Results

As J_v and L_p are qualitatively the same as a parameter of water transport, only L_p s are presented below.

Effect of DHETs on AVP response (Fig. 3). Equimolar concentrations of each DHET were used to assess their effect on AVP-stimulated osmotic water flow in CCDs. We were able to assign relative potencies to each of the four regioisomers. In AVP controls basal L_p ($\times 10^{-7}$ cm/atm per s) increased from 5.86 ± 1.6 to a peak of 234.75 ± 11.7 ($n = 17$) upon exposure of the tubules to $10 \mu\text{U}/\text{cm}^2$ vasopressin. Basal and peak L_p values were not different in tubules exposed to bath with or without vehicle (0.001% ethanol) in either control or experimental protocols. Pretreatment of CCDs with 10^{-6} M 11,12-DHET gave basal L_p measurements of 2.7 ± 1.48 , $n = 7$, NS from AVP controls. However, peak L_p in response to AVP in tubules pretreated with $1 \mu\text{M}$ 11,12-DHET was reduced to 178.56 ± 7.7 , $n = 7$, $P < 0.005$ vs. AVP controls, a reduction in peak response of 25%. 10^{-6} M 5,6-DHET resulted in a basal L_p value of 7.85 ± 1.77 , $n = 8$, again no different from control basal L_p . The 5,6-DHET pretreatment blunted peak AVP-induced L_p by 35% to 154.35 ± 11.9 , $n = 8$, $P < 0.005$. Tubules exposed to 10^{-6} M 8,9-DHET had basal L_p of 1.76 ± 1.0 and an AVP-induced peak L_p of 134.0 ± 7.97 , $n = 5$, $P < 0.0001$, a suppression from the control response of 43%. Finally, experiments using 14,15-DHET at 10^{-6} M had a basal L_p of 4.20 ± 1.3 and a peak L_p of 95.2 ± 8.39 , $n = 5$, $P < 0.0001$, a reduction of 60% from AVP controls. In these experiments we found that all four regioisomers of DHETs caused significant inhibition of the hydroosmotic effect of AVP in CCDs. 14,15-DHET had the greatest effect on vasopressin-stimulated water transport.

Dose response of 14,15-DHET (Fig. 4). The inhibitory effect of 14,15-DHET on AVP-stimulated L_p is dose dependent and significant to nanomolar concentrations. Pretreatment with 14,15-DHET at 10^{-6} M reduced the AVP-peak L_p response to 95.2 ± 8.39 , $n = 5$, $P < 0.0001$; at 10^{-7} M this value was 110.0 ± 7.22 , $n = 5$, $P < 0.0001$; at 10^{-8} M the peak L_p was

BIOLOGICAL SAMPLE + DEUTERATED STANDARDS

EXTRACT WITH CHLOROFORM / METHANOL

HPLC PURIFICATION

PURIFIED BIOLOGICAL SAMPLE

DERIVATIZE TO PFB ESTERS

HPLC PURIFICATION

NICI GC / MS

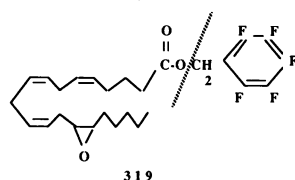


Figure 2. Scheme of methodology used to identify presence of cytochrome P450 NADPH-dependent EETs in biological samples. EETs are identified by analysis of GC retention times using selective ion monitoring during NICI GC/MS. After cleavage the EET-PFB esters share a common ion fragment at m/z 319 under NICI conditions.

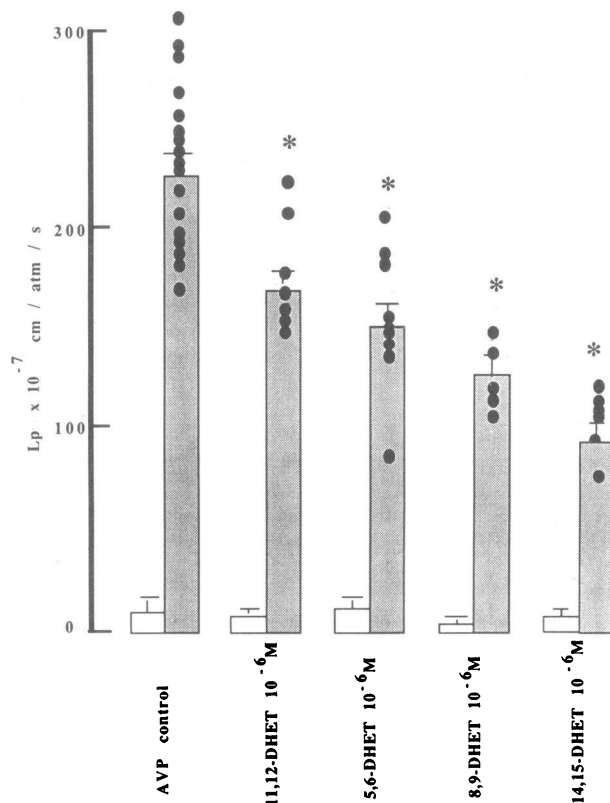


Figure 3. Effect of four regioisomeric DHETs on AVP-stimulated L_p in CCD. The diols are depicted in rank order of potency for inhibition of vasopressin-mediated water transport. Open bars, Basal L_p ; stippled bars, AVP-induced peak L_p ; dots, actual L_p values for individual experiments; *, significance of $P < 0.0005$.

130.75 ± 1.88 , $n = 4$, $P < 0.0001$; at 10^{-9} M peak L_p was 164.6 ± 10.61 , $n = 5$, $P < 0.005$. At concentrations of 10^{-10} M of 14,15-DHET, the peak L_p was 235.0 ± 4.93 , $n = 3$, NS, a response no different from AVP controls.

Comparison of 14,15-DHET vs. PGE_2 on the AVP response (Fig. 5). We used previously cited concentrations of PGE_2 used to inhibit AVP response in CCDs (26–28) to compare the inhibitory effect of 14,15-DHET on this same process. 14,15-DHET at 10^{-7} M led to a 54% inhibition of AVP peak L_p , 112.4 ± 9.94 , $n = 5$, which was not statistically different from the effect produced by equimolar PGE_2 ($P = NS$). PGE_2 at 10^{-7} M caused suppression of the AVP peak L_p to 88.8 ± 13.4 , $n = 5$, $P < 0.0001$ when compared to AVP controls, a reduction of 63%.

Effect of 14,15-DHET on CcAMP response (Fig. 6). CCDs were exposed to the permeant cAMP analogue CcAMP at 10^{-4} M. CcAMP is resistant to phosphodiesterase hydrolysis. CcAMP stimulated L_p to a peak L_p value of 189.6 ± 11 , $n = 8$. Pretreatment of tubules with 10^{-6} M 14,15-DHET reduced the peak L_p response to CcAMP to 132 ± 13.4 , $n = 5$, $P < 0.01$, a reduction of 31% from control values. These experiments indicated that DHET was, at least in part, producing its inhibition of water flow in stimulated CCDs at a step beyond cAMP generation.

Analysis of harvested CCDs for epoxygenase metabolites (Figs. 2, 7, and 8). The PFB ester derivatives isolated and purified from 550 microdissected CCDs were analyzed using capillary NICI GC/MS as described in Methods (Fig. 2). The

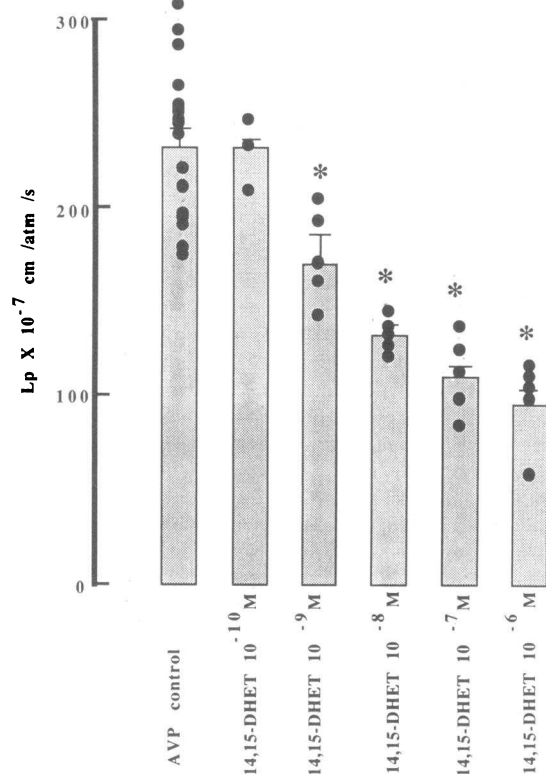


Figure 4. Dose response of 14,15-DHET on AVP-stimulated L_p . Stippled bars, AVP-induced peak L_p ; dots, L_p values for each experiment; *, significance of $P < 0.005$.

presence of EET mixtures in the biological samples was studied by comparisons of their GC and MS fragmentation properties to those of synthetic EET-PFBs. Under the conditions used, all four regioisomeric EETs coeluted from the GC column with a retention time (R_t) of ~ 11.8 min. A summary of the experimental evidence suggesting the presence of EET mixtures in the rabbit CCDs is as follows: (a) NICI GC/mass

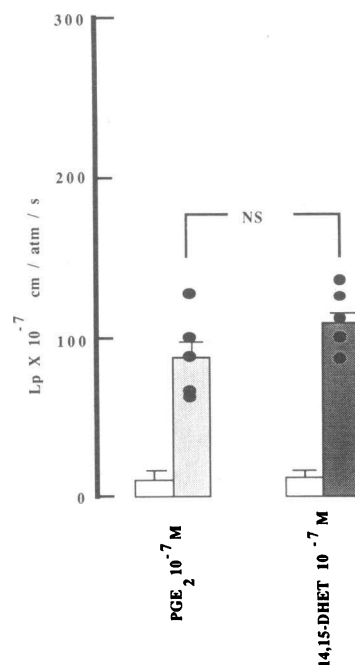


Figure 5. Inhibition of AVP-stimulated L_p by 14,15-DHET is as potent as PGE_2 . Open bars, Basal L_p ; stippled bars, experimental AVP-induced peak L_p ; dots, L_p values for each experiment.

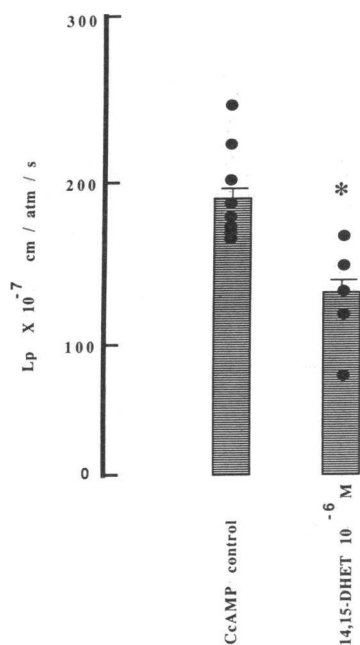


Figure 6. 14,15-DHET inhibits the osmotic water permeability response to exogenous CcAMP. Hatched bars, Peak CcAMP-induced L_p ; dots, L_p s of individual experiments; *, significance of $P < 0.01$ when 14,15-DHET experimental L_p s are compared with control CcAMP peak L_p s.

fragmentographic analysis of an aliquot of the PFB ester derivative of the biological sample shows coelution of the EET-PFB-derived ion fragments at mass/ionic charge ratio (m/z) 319 (loss of PFB), 303 (loss of PFB and oxygen), and 301 (loss of PFB and water) with an R_f similar to that of an equimolar mixture of all four synthetic EET-PFBs (11.8 min) as shown in Fig. 7 A). The mass spectrum of the material present in the biological sample and elution from the GC column at 11.8 min shows the typical EET-PFB-derived ions at m/z 319 (base peak) and 303. With the exception of a contaminant ion fragment at m/z 311, the fragmentation pattern shown is almost identical to published EET-PFB spectra (Fig. 7 B; 8). Coinjection of an aliquot of the biological material with a known quantity of synthetic [5,6,8,9,11,12,14,15- 2H]14,15-EET-PFB shows the presence in the mixture of the EET-PFB-derived ion fragments at m/z 319, 303, and 301, and of an ion fragment at m/z 327, derived from the loss of PFB from the octadeuterated standard (Fig. 8 B). The slight difference in retention times (6–7 s) between the protium and octadeuterated EET-PFB esters is due to well-established isotopic effects. The absence of contaminant ion fragments at m/z 319, 303, and 301 in our synthetic [2H_8]14,15-EET-PFB is illustrated by the mass spectrum of the material shown in Fig. 8 A.

Discussion

Oxygenated metabolites of AA are important modulators of cellular biochemistry and physiology. Much is known about cyclooxygenase metabolites. Less well examined are the arachidonate metabolites produced by lipoxygenases and cytochrome P450 NADPH-dependent epoxygenase. Since biologic activity has been demonstrated for cytochrome P450 epoxygenase metabolites of arachidonate in certain cells, we determined that the physiologic role they may fulfill in the kidney should be further studied.

The model system that we chose was a hormonally responsive epithelium, the rabbit CCD perfused *in vitro*. The CCD is water impermeable under basal conditions but responds to

vasopressin by becoming permeable to osmotically driven water flow. This hydroosmotic effect of AVP is accomplished at the intracellular level through generation of the second messenger, cAMP (26–30).

The present studies demonstrate a specific biologic action of all four regioisomers of DHETs. DHETs are potent inhibitors of the hydroosmotic effect of AVP in the mammalian CCD. However, equimolar concentrations of the separate isomers of DHET vary in the degree to which they suppress the response to AVP in this nephron segment. We find a rank order of increasing inhibitory effect as follows: 11,12-DHET < 5,6-DHET < 8,9-DHET < 14,15-DHET (Fig. 3). 14,15-DHET, the most potent epoxygenase-derived diol, blunts vasopressin's action in a dose-dependent fashion: by 60% at micromolar concentrations and 31% at nanomolar concentrations (Fig. 4).

The hydroosmotic effect of vasopressin in CCD is modulated by cyclooxygenase products, PGs of the E series (2, 4, 26–30). The current view is that PGs act at the level of a guanine nucleotide inhibitory regulatory protein that inhibits activation of adenylate cyclase and thereby inhibits generation of the second messenger cAMP (28–31). Vasopressin occupancy of its cell surface receptor causes a guanine nucleotide stimulatory regulatory protein to interact with the catalytic subunit of adenylate cyclase and promote endogenous cAMP formation. Exogenous cAMP or cAMP analogues like vasopressin stimulate osmotic water flow in CCDs (26–30). Exogenous or endogenous PGs inhibit the response to vasopressin in *in vitro* studies (27–30). At equimolar concentrations 14,15-DHET caused suppression of AVP-stimulated water transport comparable to the suppression seen with PGE₂ (Fig. 5). In contrast to studies reporting no effect of PGE on exogenous cAMP-mediated water flow, we found that DHET inhibited the permeability response to CcAMP (Fig. 6). 14,15-DHET was not as potent in suppressing water flow in response to CcAMP as it was in inhibiting the AVP response.

Our GC/MS data as well as previously published material strongly suggest the presence of epoxygenase products as endogenous constituents of rabbit kidney CCDs. The CCDs used for NICI GC/MS analysis were harvested with the same method used to isolate tubules for our microperfusion experiments. Similar techniques have been used to demonstrate the presence of 8,9-EET and 14,15-EET in whole rabbit kidneys (8) and to detect the presence of these same epoxides in human urine (24). Unfortunately, in the present case the limited quantity of material precluded a more conclusive analysis. While we have preliminary data to document endogenous EETs in CCDs, their specific location is unknown at present. However, in rat liver EETs are identified in phosphatidylinositol and phosphatidylethanolamine esterified to the *sn*-2 position of the glycerol backbone of these phospholipids (25). This finding may be important given that biologically active metabolites of arachidonate are classically felt to depend on metabolism of free AA generated through the action of phospholipase A₂ on cellular phospholipids (32). Biologically active epoxygenase products present in cell membrane phospholipids could be released by the action of either phospholipase A₂ or phospholipase C.

Schlondorff et al. recently reported studies concerning the effect of EETs and DHETs on vasopressin-induced water flow in amphibian urinary bladder, an epithelium that shares many of the transport functions of the mammalian collecting duct

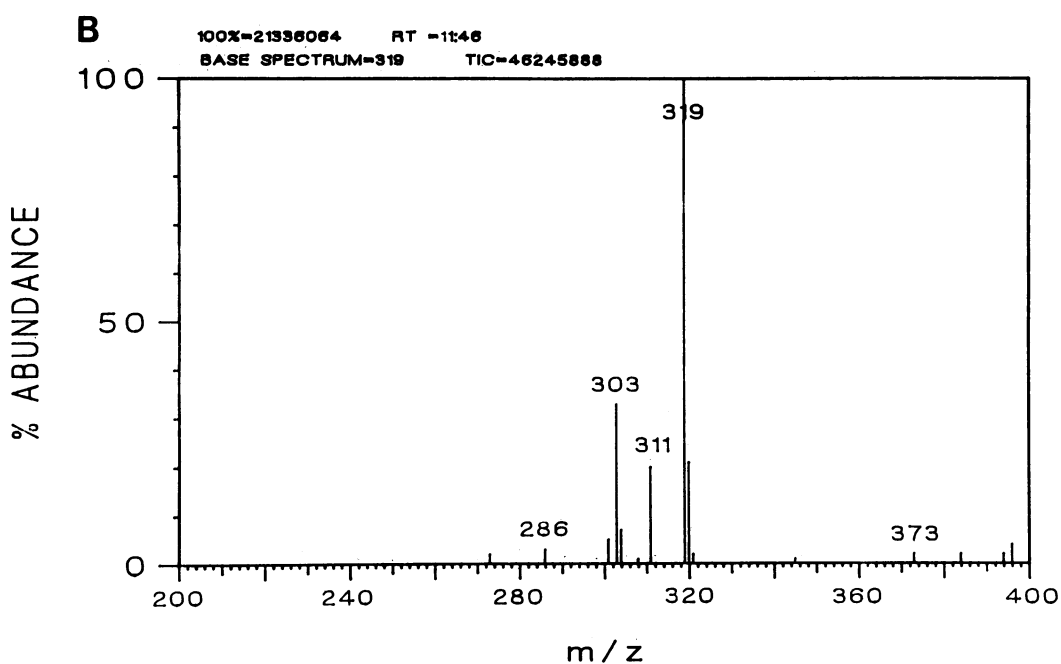
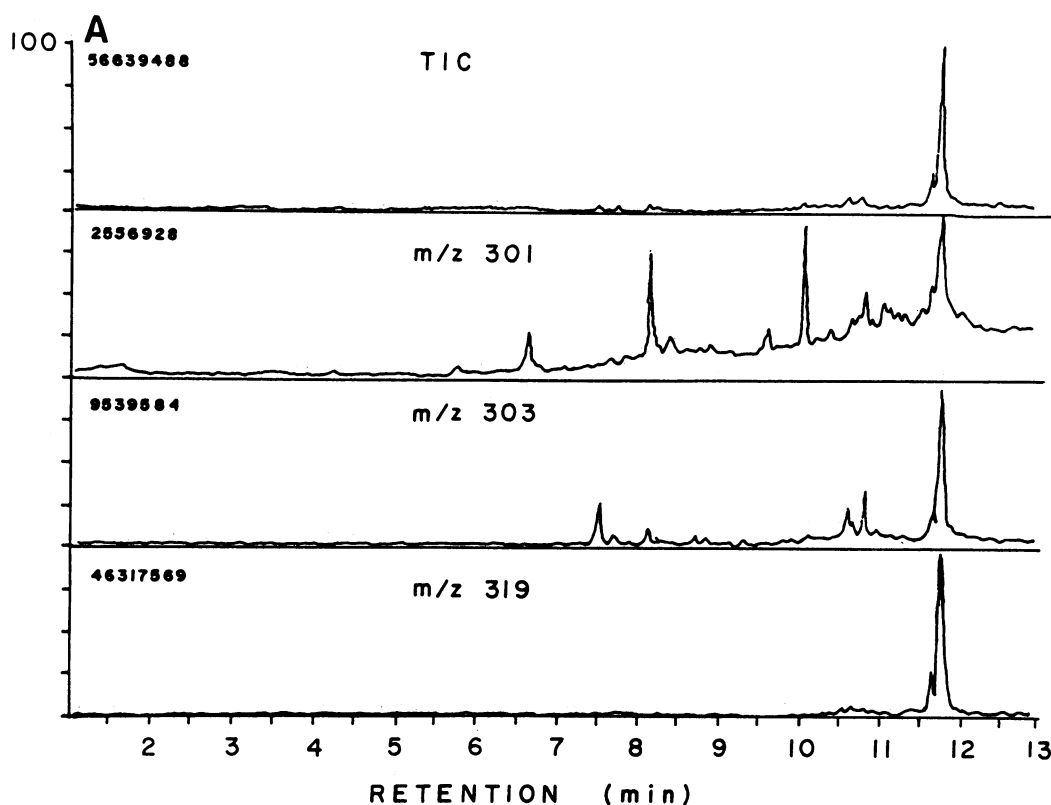


Figure 7. NICI GC/MS analysis of the sample extracted and purified from rabbit kidney CCD. An aliquot of the PFB derivative of the biological sample was injected into a 30-m SPB-5 fused silica capillary column. Column eluents were monitored using NICI MS as described in Methods. *A*, Mass fragmentographic analysis of the GC column eluent performed by selective ion monitoring at m/z 319 (loss of PFB), 303 (loss of PFB and oxygen), and 301 (loss of PFB and water). *Abcissa*, R_t in minutes; *ordinate*, percent abundance. *B*, Mass spectrum of the material eluting with a GC retention time similar to that of synthetic 14,15-EET-PFB (11 min, 46 s, or 11.8 min). *Abcissa*, m/z ; *ordinate*, percent abundance.

(18). Their study also reports significant inhibitory activity for EETs and DHETs on AVP-mediated water transport. Experiments were performed at room temperature using mounted toad hemibladders. 11,12-EET and 5,6-EET and their respective diols (DHETs) produced inhibition of the vasopressin response. However, to achieve significance, concentrations of 10^{-6} – 10^{-5} M of these compounds were required. EETs and DHETs also inhibited vasopressin-induced adenylate cyclase activity. The inhibitory effect on AVP's action by 11,12-EET was comparable to that seen with PGE_2 . 11,12-EET, as op-

posed to the other isomers, also reduced osmotic water flow in response to 8-bromo-3',5' cAMP. These authors proposed a mechanism of action for EETs and DHETs on AVP activity through an inhibition of AVP-induced cAMP generation (18). Experiments presented in this communication differ importantly from the studies of Schlondorff et al. in the following ways: (a) our experiments were performed in a mammalian system at 37°C ; (b) the concentrations of eicosanoid used were more likely to be physiologically relevant (the concentration of EETs in normal, pooled human urine was ~ 2 nM; see refer-

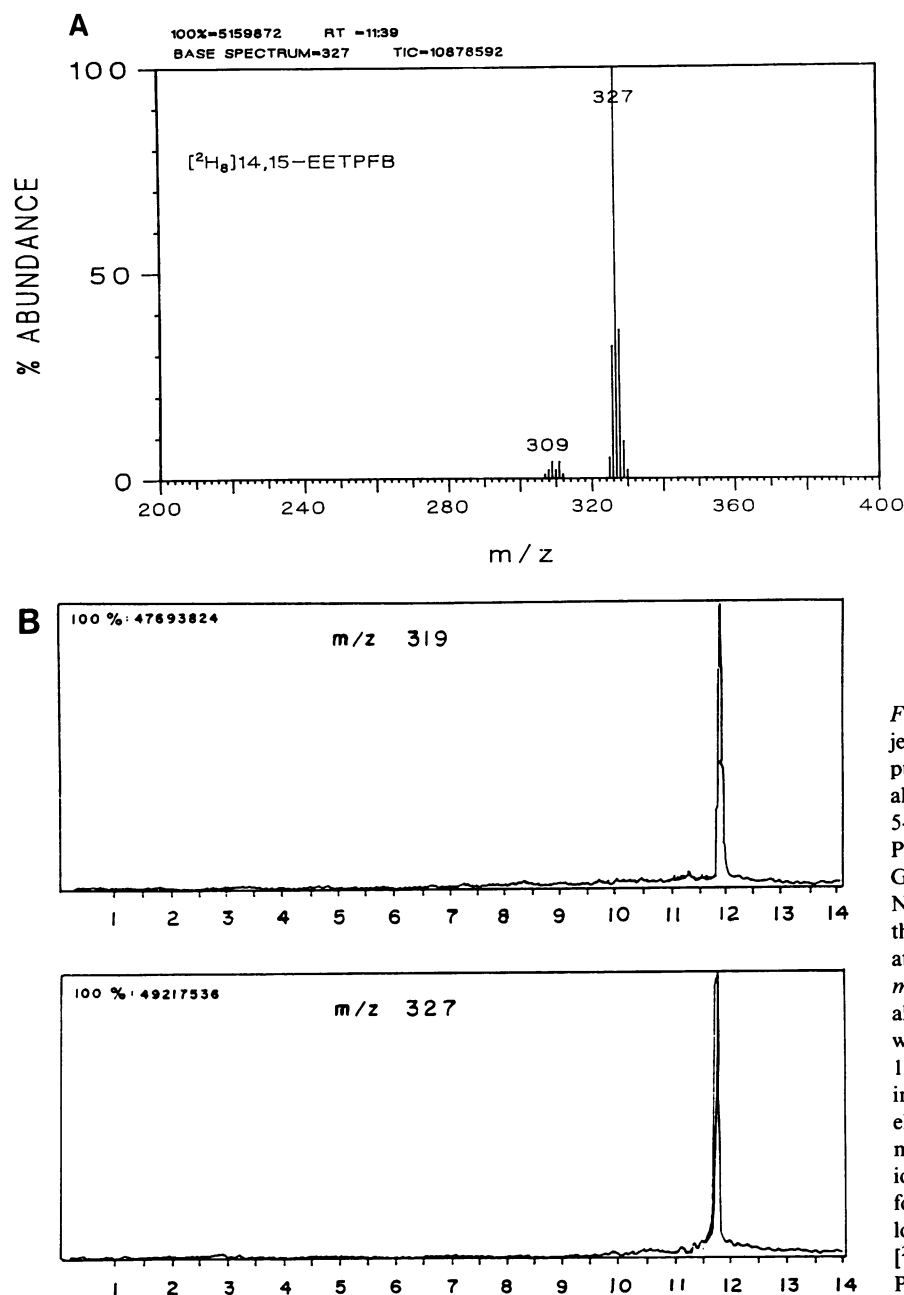


Figure 8. NICI GC/MS analysis of a coinjected mixture of the sample extracted and purified from rabbit kidney CCD and an aliquot of synthetic $[^2H_8]14,15\text{-EET}$. **A**, A 5-ng sample of synthetic $[^2H_8]14,15\text{-EET-PFB}$ ester was injected into the capillary GC column and the eluent monitored by NICI MS. Shown is the mass spectrum of the material eluting from the GC column at ~ 11 min, 39 s or 11.65 min. *Abcissa*, m/z ; *ordinate*, percent abundance. **B**, An aliquot of the biological sample was mixed with a known quantity of synthetic $[^2H_8]14,15\text{-EET-PFB}$ (1 ng) and then injected into the capillary GC column. The column eluent was monitored by NICI mass fragmentationography at m/z 319 and 327. Selected ion current profile under NICI conditions for the EET-PFB ester present in the biological sample, m/z 319 (M-PFB); and $[^2H_8]14,15\text{-EET-PFB}$ ester, m/z 327 (M-PFB).

ence 24); and (c) our data indicating that DHET inhibits the osmotic water flow induced by exogenous cAMP suggests that epoxigenase metabolites may influence water transport through some process other than, or in addition to, reduction of adenylate cyclase activity.

Determination of the mechanism whereby cytochrome P450 metabolites of arachidonate inhibit water transport in CCD will require future experiments. To address any component of DHET action that involves cAMP generation will require measurements of cAMP production. It has been shown that calcium and calcium-activated, phospholipid-dependent protein kinase C inhibit the hydroosmotic effect of vasopressin in CCD and amphibian urinary bladder (33–36). Importantly, Ando et al. (33) demonstrated that protein kinase C activation leads to an inhibition of vasopressin-mediated water transport through a post-cAMP process. Thus it is possible that the DHETs transduce their inhibitory effect through changes in

cell calcium or activation of protein kinase C. Support for this notion is derived from studies demonstrating that EETs affect calcium binding, uptake, and release from microsomes made from vascular smooth muscle (35). This hypothesis remains to be tested.

In summary, we have presented evidence that DHETs produced by cytochrome P450 NADPH-dependent epoxigenase are potent inhibitors of the hydroosmotic effect of vasopressin in rabbit CCD. The 14,15-DHET isomer evokes the most potent suppressive action, and the inhibition is dose dependent. DHET is as potent as PGE_2 in inhibiting AVP-mediated water transport, a process thought to be mediated via cAMP. We also have shown that these P450 metabolites of AA are present in the normal CCD. Finally, although the specific mechanism(s) by which these compounds produce their inhibitory effect will require further study, their inhibition of vasopressin action in the CCD involves a post-cAMP step.

Acknowledgments

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