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Arachidonic Acid Inhibits Epithelial Na Channel Via Cytochrome P450 (CYP) Epoxygenase-dependent Metabolic Pathways

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ABSTRACT We used the patch-clamp technique to study the effect of arachidonic acid (AA) on epithelial Na channels (ENaC) in the rat cortical collecting duct (CCD). Application of 10 μM AA decreased the ENaC activity defined by NPo from 1.0 to 0.1. The dose–response curve of the AA effect on ENaC shows that 2 μM AA inhibited the ENaC activity by 50%. The effect of AA on ENaC is specific because neither 5,8,11,14-eicosatetraynoic acid (ETYA), a nonmetabolized analogue of AA, nor 11,14,17-eicosatrienoic acid mimicked the inhibitory effect of AA on ENaC. Moreover, inhibition of either cyclooxygenase (COX) with indomethacin or cytochrome P450 (CYP) ω-hydroxylation with *N*-methylsulfonyl-12,12-dibromododec-11-enamide (DDMS) failed to abolish the effect of AA on ENaC. In contrast, the inhibitory effect of AA on ENaC was absent in the presence of *N*-methylsulfonyl-6-(propargyloxyphenyl)hexanamide (MS-PPOH), an agent that inhibits CYP-epoxygenase activity. The notion that the inhibitory effect of AA is mediated by CYP-epoxygenase–dependent metabolites is also supported by the observation that application of 200 nM 11,12-epoxyeicosatrienoic acid (EET) inhibited ENaC in the CCD. In contrast, addition of 5,6-, 8,9-, or 14,15-EET failed to decrease ENaC activity. Also, application of 11,12-EET can still reduce ENaC activity in the presence of MS-PPOH, suggesting that 11,12-EET is a mediator for the AA-induced inhibition of ENaC. Furthermore, gas chromatography mass spectrometry analysis detected the presence of 11,12-EET in the CCD and CYP2C23 is expressed in the principal cells of the CCD. We conclude that AA inhibits ENaC activity in the CCD and that the effect of AA is mediated by a CYP-epoxygenase–dependent metabolite, 11,12-EET.

KEY WORDS: Na reabsorption • ENaC • epoxyeicosatrienoic acid • cortical collecting duct • MS-PPOH

INTRODUCTION

Maintaining Na homeostasis is essential for the regulation of blood pressure (Verrey et al., 2000). The kidney plays an important role in maintaining the extracellular Na in the normal range (Koeppen and Stanton, 1992) and the cortical collecting duct (CCD) is responsible for the hormone-regulated Na absorption. Na absorption in the CCD takes place by a two-step process: Na enters the cells across the apical membrane via ENaC (epithelial Na channel) and is extruded from the cell across the basolateral membrane through Na-K-ATPase. It is well established that the rate-limiting step for Na absorption in the CCD is the apical Na conductance (Koeppen and Stanton, 1992), which is regulated by aldosterone and Na intake; a high aldosterone level augments, whereas an increased Na intake decreases, apical ENaC channel activity. Moreover, it has been demonstrated that the early effect of aldosterone on ENaC is to stimulate the channel open probability (Kemendy et al., 1992), whereas the late effect of aldosterone (>1 h) is to increase the ENaC number in the apical membrane (Pácha et al., 1993). Although decreases in plasma aldosterone levels play a role in suppressing renal Na transport induced by high Na intake (Verrey et al., 2000), it is possible that factors other than aldosterone may also be involved in the inhibition of renal Na transport by high Na intake. Relevant to this hypothesis is the observation that high Na intake increases cytochrome P450 (CYP)-epoxygenase–dependent arachidonic acid (AA) metabolites such as 11,12-epoxyeicosatrienoic acid (EET) (Makita et al., 1994). This suggests that AA and its metabolites may be involved in the regulation of Na transport in the CCD. In this regard, it has been reported that 5,6-EET inhibits Na transport in the rabbit CCD (Sakairi et al., 1995). AA has also been shown

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Abbreviations used in this paper: AA, arachidonic acid; AQP2, aquaporin 2; CCD, cortical collecting duct; COX, cyclooxygenase; CYP, cytochrome P450; DDMS, *N*-methylsulfonyl-12,12-dibromododec-11-enamide; EA, eicosatrienoic acid; EET, epoxyeicosatrienoic acid; ENaC, epithelial Na channel; ETYA, 5,8,11,14-eicosatetraynoic acid; 20-HETE, 20-hydroxyeicosatetraenoic acid; MS-PPOH, *N*-methylsulfonyl-6-(propargyloxyphenyl)hexanamide.

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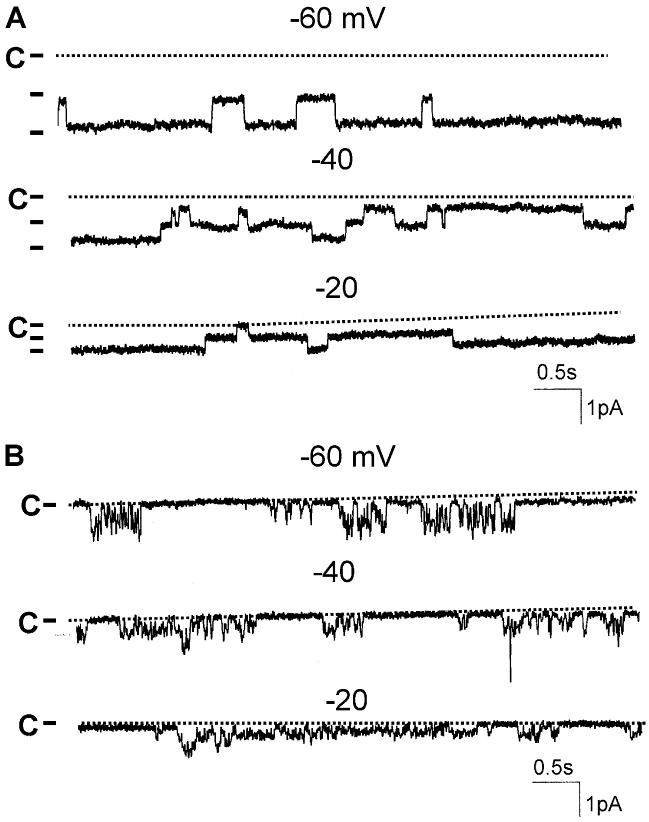

FIGURE 1. A channel recording showing the channel activity of ENaC under control conditions (without amiloride) (A) and in the presence of 0.5 μM amiloride (B). The channel closed level is indicated by "C" and the holding potential was indicated on the top of the corresponding trace.

to block the apical 35 pS K channel in the apical membrane of the CCD, however, it is not clear whether AA can also regulate the ENaC activity. Therefore, the present study is designed to examine the effect of AA on ENaC and to determine the metabolite responsible for the effect of AA on ENaC.

MATERIALS AND METHODS

Preparation of CCDs

Pathogen-free Sprague-Dawley rats of either sex (5–6 wk) were used in the experiments and purchased from Taconic Farms, Inc. Rats were maintained on a Na-deficient diet for 5–7 d and killed by cervical dislocation. Kidneys were removed immediately and several thin slices of the kidney (<1 mm) were cut. The kidney slices were placed on an ice-cold Ringer solution and the CCD was isolated with watch-make forceps. The isolated CCD was placed on a 5 × 5 mm coverglass coated with polylysine and the coverglass was transferred to a chamber (1,000 μl) mounted on an inverted Nikon microscope. The CCDs were superfused with HEPES-buffered NaCl solution, and the temperature of the chamber was maintained at 37 ± 1°C by circulating warm water surrounding the chamber. The CCD was cut open with a sharpened micropipette to expose the apical membrane. For the measurement of 11,12-EET, both kidneys were perfused with 0.5% collagenase-containing ringer to wash out the blood. The kidney slices were incubated with the 0.5% collagenase-containing Ringer for an additional 3–5 min before dissection.

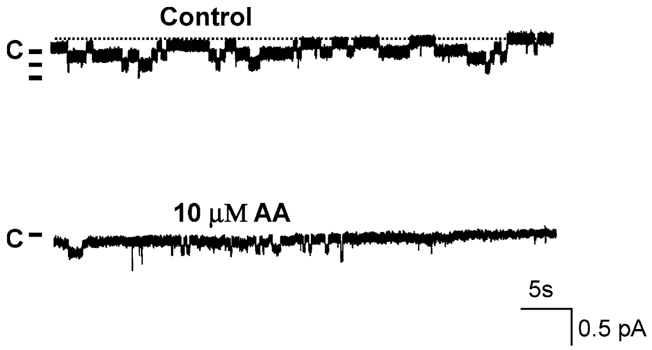

FIGURE 2. A channel recording showing the effect of AA on ENaC in a cell-attached patch. The channel closed level is indicated by "C" and a dotted line. The holding potential was −40 mV (hyperpolarization).

Patch-clamp Technique

An Axon200A patch-clamp amplifier was used to record channel current that was low-pass filtered at 50 Hz by an eight pole Bessel filter (902LPF; Frequency Devices). The Na current was recorded and digitized by an Axon Instruments, Inc. interface (Digidata 1200). Data were analyzed using the pClamp software system 7.0 (Axon Instruments, Inc.). Channel activity defined as NP_o was calculated from data samples of 60 s duration in the steady state as follows:

$$NP_o = \sum(t_1 + 2t_2 + \ldots it_i), \tag{1}$$

where t_i was the fractional open time spent at each of the observed current levels. The channel conductance was calculated by recording the current at least three holding potentials.

Measurement of EET

The CCDs were isolated and placed in a tube containing ice-cold Na Ringer (0.5 ml). Eicosanoids in the CCD and media were acidified to pH 4.0 with 9% formic acid. After addition of 2 ng of D_8 11,12-EET as internal standard, the samples were extracted twice with 2× volume ethyl acetate and evaporated to dryness. After extraction, the tubules were homogenized and the protein concentration was measured. The samples were purified by reverse phase (RP)-HPLC on a C_{18} μBondapak column (4.6 × 24 mm) using a linear gradient from acetonitrile:water:acetic acid (62.5:37.5:0.05%) to acetonitrile (100%) over 20 min at a flow rate of 1 ml/min. The fraction containing 11,12-EET was collected on the basis of the elution profile of standards monitored by ultraviolet absorbance (205 nm). The fractions were evaporated to dryness and resuspended in 100 μl of acetonitrile. HPLC fractions containing 11,12-EET were derivatized as described earlier (Croft et al., 2000). The derivatized 11,12-EET was dried with nitrogen and resuspended in 50 μl of iso-octane for gas chromatography mass spectrometry analyses. A 1-μl aliquot of derivatized CYP-derived AA metabolites, dissolved in iso-octane, was injected into a GC (Hewlett Packard 5890) column (DB-1ms; 10.0 m, 0.25 mm inner diameter, 0.25 μm film thickness; Agilent). We used temperature programs ranging from 150 to 300°C at rates of 25°C/min, respectively (Macica et al., 1993). Methane was used as a reagent gas at a flow resulting in a source pressure of 1.3 torr and the MS (Hewlett-Packard 5989A) was operated in

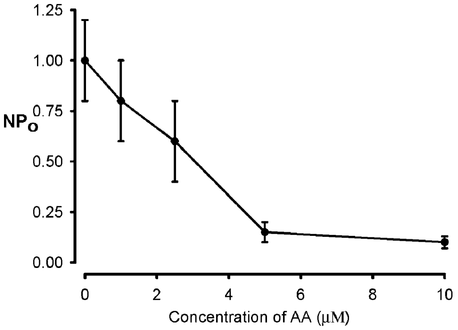

FIGURE 3. A dose–response curve of AA effect on ENaC. Each point represents a mean value from 3–10 patches.

electron capture chemical ionization mode. The endogenous 11,12-EET (ion *m/z* 319) was identified by comparison of GC retention times with authentic D_8 11,12-EET (*m/z* 327) standards.

Immunocytochemical Staining

Sprague-Dawley rats (6 wk) were anesthetized with isoflurane and the abdomen was cut open for perfusion of kidneys with 50 ml PBS containing heparin (40 unit/ml) followed by 200 ml of 4% paraformaldehyde. After perfusion, the kidneys were removed and subjected to post-fixation with 4% paraformaldehyde for 12 h. The kidneys were dehydrated and cut to 8–10 μM slices by Leica1900 cryostat (Leica). The tissue slices were dried at 42°C for 1 h. The slides were washed with 1× PBS for 15 min, and permeabilized with 0.4% Triton dissolved in 1× PBS buffer containing 1% BSA and 0.1% lysine (pH 7.4) for 15 min. Kidney slices were blocked with 2% goat serum for 1 h at room temperature and then incubated with aquaporin 2 (AQP2) (Alomone) and CYP2C23 (a gift from J. Capdevila, Vanderbilt University, Nashville, TN) for 12 h at 4°C. Slides were thoroughly washed with 1× PBS followed by incubation in second antibody mixtures in 0.4% Triton dissolved in 1× PBS for 2 h at room temperature.

Solution and Statistics

The bath solution contains (in mM) 140 NaCl, 5 KCl, 1.8 $CaCl_2$, 1.8 $MgCl_2$, and 10 HEPES (pH 7.4). The pipette solution was composed of (in mM) 135 NaCl, 5 KCl, 1.8 Mg_2Cl, and 5 HEPES (pH 7.4). Indomethacin was purchased from Sigma-Aldrich whereas 5,8,11,14-eicosatetraynoic acid (ETYA) and EETs were obtained from Biomol. AA and 11,14,17-eicosatrienoic acid were obtained from Nu-Check. *N*-methylsulfonyl-12,12-dibromododec-11-enamide (DDMS) and *N*-methylsulfonyl-6-(propargyloxyphenyl)hexanamide (MS-PPOH) were synthesized in Dr. Falck's laboratory, Southwestern Medical Center at Dallas. DDMS and MS-PPOH are AA analogs which inhibit CYP-dependent ω-hydroxylation and CYP-epoxygenase, respectively (Wang et al., 1998). The data is presented as mean ± SEM. We used paired and unpaired Student's *t*-test to determine the statistical significance. If the P value was less than 0.05, the difference was considered to be significant.

RESULTS

AA Inhibits ENaC

We used the patch-clamp technique to study the effect of AA on ENaC in the isolated CCD from rats on a Na-deficient diet (<0.001%) for 5–7 d because ENaC activity is very difficult to detect in the CCD from rats on a normal Na diet (0.5%). Fig. 1 A is a recording showing ENaC activity in a cell-attached patch at −60, −40, and −20 mV (hyperpolarization), respectively. The channel slope conductance is 5.5 ± 0.5 pS between −80 and −20 mV. Fig. 1 B shows the channel activity in the presence of 0.5 μM amiloride in the pipette solution. It is apparent that the channel activity is flickery, an indication of amiloride-mediated blockade. To study the effect of AA on ENaC, we recorded the channel activity under control conditions for 3–5 min. When Na channel activity reached a steady state, AA was applied to the bath media while the channel activity was continuously monitored. The effect of AA can be observed in 5 min approximately and it reaches the steady state in 10–15 min. Thus, we have selected a 60-s-long record in the steady state between 10–15 min after AA to calculate NP_o. Fig. 2 is a recording showing that application of 10 μM AA inhibited ENaC and reduced NP_o from 1.0 ± 0.2 to 0.1 ± 0.03 ($n = 10$). The effect of AA was reversible because washout of AA restored the channel activity (unpublished data). Since the experiments were performed in the split-open tubule, it is not possible to determine the sidedness of AA effect.

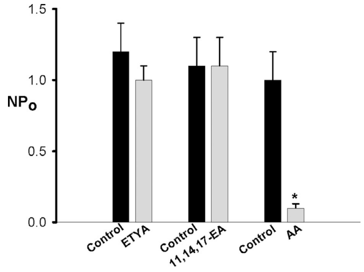

FIGURE 4. Effect of 10 μM ETYA, 11,14,17-EA, and AA on ENaC activity. The experiments were performed in cell-attached patches.

After establishing that AA inhibits ENaC, we studied the dose response curve of AA effect on ENaC in the CCD. Fig. 3 is a dose–response curve of the AA effect on ENaC and K_d, a concentration that is required to inhibit the channel activity by 50%, is ~2 μM.

To determine whether the effect of AA is the result of changing membrane fluidity that could affect channel activity (Meves, 1994; Petrou et al., 1994), we also examined the effect on ENaC of ETYA, a nonmetabolizable AA analogue, and 11,14,17-eicosatrienoic acid (EA), an unsaturated fatty acid. We followed the protocol of AA experiments and examined the ENaC activity in the continuous presence of ETYA or EA for at least 10 min. Data summarized in Fig. 4 show that application of ETYA (10 μM) (control NP_o, 1.2 ± 0.2; ETYA, 1.0 ± 0.1) ($n = 5$) and 10 μM EA failed to inhibit the activity of ENaC (control NP_o, 1.1 ± 0.2, EA, 1.1 ± 0.2) ($n = 5$). This indicates that the inhibitory effect of AA on ENaC is not the result of altering the membrane physical properties.

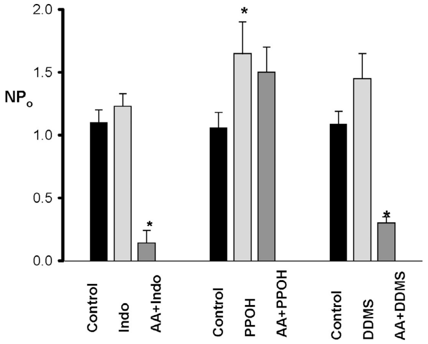

FIGURE 5. The effect of 10 μM AA in the presence 5 μM indomethacin (Indo), 15 μM MS-PPOH (PPOH), and 5 μM DDMS. The experiments were performed in cell-attached patches and asterisk indicates that the data is significantly different from the corresponding control value.

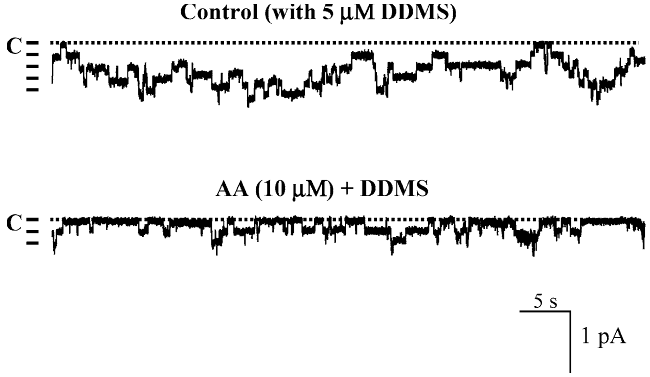

FIGURE 6. A channel recording showing the effect of 10 μM AA on ENaC in the presence DDMS. The channel closed level is indicated by a dotted line.

The Inhibitory Effect of AA Is Mediated by CYP-epoxygenase–dependent Pathway

After demonstrating that AA inhibited ENaC in the CCD, we examined whether the effect of AA on ENaC was mediated by AA per se or AA metabolites. Thus, we extended the study by examining the effect of AA on ENaC in the presence of specific inhibitors of AA metabolic enzymes. It is well established that AA can be metabolized by cyclooxygenase (COX)-dependent pathway in the renal tubules. To test the possibility that AA inhibits ENaC by COX-dependent AA metabolites, we examined the effect of AA in the presence of indomethacin, an inhibitor of COX. Application of 5 μM indomethacin had no effect on ENaC activity (control NP$_o$, 1.1 ± 0.1; indomethacin, 1.23 ± 0.1) ($n = 6$) (Fig. 5). Moreover, inhibition of COX failed to abolish the AA-induced inhibition on ENaC. Data summarized in Fig. 5 show that in the presence of indomethacin, application of AA still inhibited the ENaC activity (NP$_o$, 0.14 ± 0.1) ($n = 6$). This indicates that the effect of AA on ENaC was not mediated by COX-dependent metabolites. Also, we investigated the effect of AA on ENaC in the presence of inhibitors of CYP monooxygenases, which have been shown to be expressed in the kidney (McGiff, 1991). First, we tested whether inhibition of CYP ω-hydroxylase could abolish the inhibitory effect of AA on ENaC. We have previously shown that AA is converted to 20-hydroxyeicosatetraenoic acid (20-HETE) by CYP-dependent ω-hydroxylation and that 20-HETE inhibits the apical 70 pS K channel in the TAL (Wang and Lu, 1995). Therefore, we examined the effect of AA on ENaC in the presence of DDMS, an inhibitor of CYP-dependent ω-hydroxylation (Wang et al., 1998). Inhibition of CYP hydroxylases did not significantly alter the channel activity (control NP$_o$, 1.1 ± 0.1; DDMS, 1.45 ± 0.2) ($n = 10$). Also, AA can still inhibit the channel activity in the presence of DDMS. Fig. 6 is a recording showing that DDMS failed to abolish the inhibitory effect of AA on ENaC and that 10 μM AA reduced NP$_o$ from 1.45 ± 0.2 to 0.3 ± 0.05 ($n = 10$).

After excluding the role of CYP-dependent hydroxylation in mediating the effect of AA on ENaC, we examined the effect of AA on ENaC in the presence of MS-PPOH, an inhibitor of CYP-epoxygenase (Wang et al., 1998). Blocking CYP-epoxygenase not only significantly increased ENaC activity from 1.06 ± 0.12 to 1.65 ± 0.25 ($n = 11$) but also abolished the effect of AA on ENaC. Fig. 7 is a representative recording demonstrating that inhibition of CYP-epoxygenase abolished the effect of AA on ENaC because NP$_o$ in the presence of AA was 1.5 ± 0.2, which is not significantly different from that without AA.

11,12-EET Inhibits ENaC

After determining that the inhibitory effect of AA on ENaC is mediated by the epoxygenase-dependent pathway, we investigated the effect of 11,12-EET on ENaC because 11,12-EET accounts for 60% of the total EET

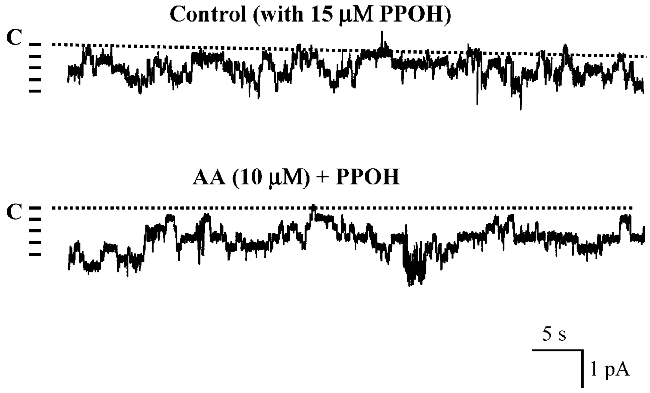

FIGURE 7. A channel recording showing the effect of 10 μM AA on ENaC in the presence of MS-PPOH. The channel closed level is indicated by a dotted line.

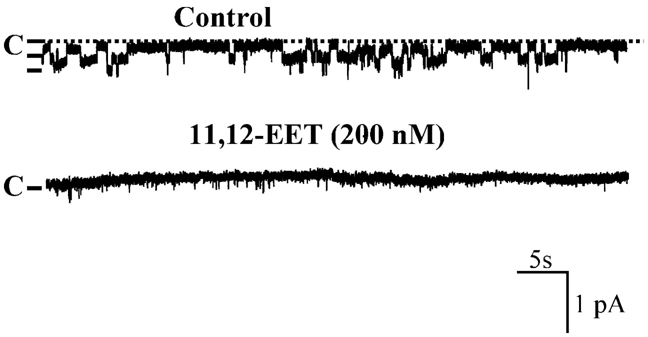

Figure 8. A channel recording showing the effect of 200 nM 11,12 EET on ENaC. The experiment was performed in a cell-attached patch and the channel closed level is indicated by a dotted line.

in the kidney (Holla et al., 1999). Fig. 8 is a channel recording made in a cell-attached patch. It is apparent that application of 200 nM 11,12-EET mimicked the effect of AA and inhibited the activity of ENaC. Data summarized in Fig. 9 demonstrate that 200 nM 11,12-EET reduced NP_o from 1.2 ± 0.12 to 0.2 ± 0.05 ($n = 9$). In contrast, addition of 200 nM 20-HETE did not block the ENaC activity (Fig. 9) and NP_o was not changed before (1.05 ± 0.1) and after 20-HETE (1.0 ± 0.1). This strongly suggests that CYP-epoxygenase plays a role in the regulation of ENaC in the CCD. To test whether the effect of 11,12-EET on ENaC could be mimicked by other EETs such as 5,6-EET, we examined the effects of 5,6-EET, 8,9-EET, and 14,15-EET on ENaC in the CCD. Data summarized in Fig. 9 demonstrated that application of 200 nM 5, 6-EET (control NP_o, 1.3 ± 0.17; EET, 1.0 ± 0.18) ($n = 5$), 8,9-EET (control NP_o, 1.4 ± 0.19; EET, 1.04 ± 0.2) ($n = 5$), or 14,15-EET (control NP_o, 0.92 ± 0.17; EET, 0.77 ± 0.2) ($n = 5$) did not significantly decrease the ENaC activity.

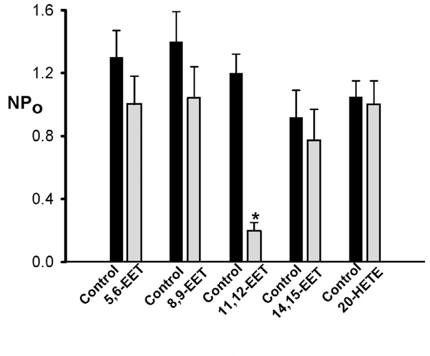

Figure 9. The effect of 200 nM 5,6-, 8,9-, 11,12-, 14,15-EET and 20-HETE on ENaC. The asterisk indicates that the difference is significant from the corresponding control value. Experiments were performed in cell-attached patches.

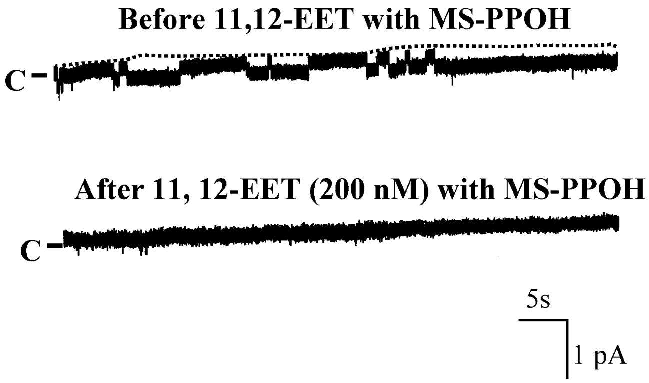

Figure 10. A channel recording showing the effect of 200 nM 11,12-EET on ENaC in the presence MS-PPOH. The channel closed level is indicated by a dotted line and the experiment was conducted in a cell-attached patch.

The view that 11,12-EET is responsible for the AA-induced inhibition of ENaC is further supported by the observation that the inhibitory effect of 11,12-EET on ENaC was also observed in the presence of MS-PPOH (Fig. 10) and decreased NP_o from 1.1 ± 0.1 to 0.12 ± 0.03 ($n = 6$).

EET Is Present and CYP2C23 Is Expressed in the CCD

After demonstrating that AA inhibits ENaC and that this effect of AA can be mimicked by 11,12-EET, we examined whether 11,12-EET is present in the CCD. The CCDs were isolated and 11,12-EET extracted from the CCDs was measured by GCMS. Fig. 11 is a histogram showing that 11,12-EET is present in the isolated CCD and the mean value from four measurements was 3.06 ± 0.3 ng/mg protein. Because CYP2C23 is the principal isoform of CYP-epoxygenase responsible for converting AA to 11,12-EET (Muller et al., 2004), we examined whether CYP2C23 is expressed in the CCD using confocal microscope. Fig. 12 is a typical confocal image showing that CYP2C23 is expressed in the AQP2-positive cells in the CCD. This confirms that CYP2C23 is present in principal cells of the CCD.

DISCUSSION

In the present study, we have demonstrated that AA inhibits ENaC in the CCD. Two lines of evidence indicate that the inhibitory effect of AA is not the result of changing membrane fluidity: (1) neither ETYA nor 11,14,17-eicosatrienoic acid had a significant effect on ENaC; and (2) the effect of AA on ENaC was absent in the presence of MS-PPOH, an inhibitor of CYP-epoxygenase.

AA at high concentrations (50 µM) has been shown to inhibit the ENaC activity in *Xenopus* oocytes expressing three ENaC subunits (α, β, and γ) and the effect of AA is the result of stimulating the endocytosis of ENaC (Carattino et al., 2003). Two lines of evidence suggest that 50 µM AA-induced inhibition of ENaC is not the result of stimulating CYP-epoxygenase–dependent AA

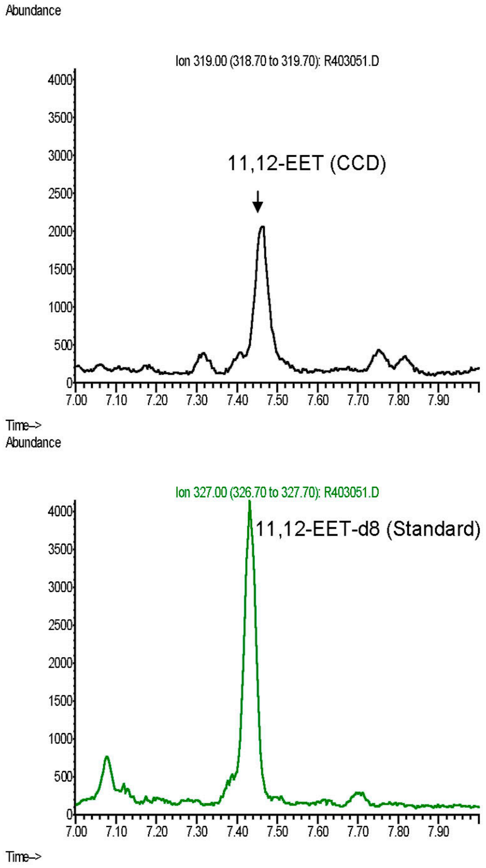

Figure 11. A histogram showing the presence of 11,12-EET in the isolated CCD (top). The standard of 11,12-EET is shown on the bottom of the figure.

metabolism. First, ETYA, a nonmetabolizable AA analogue, mimicked the effect of 50 μM AA on ENaC activity in oocytes. In contrast, application of ETYA failed to inhibit ENaC activity in the CCD. Also, the finding that blockade of CYP-epoxygenase abolished the effect of AA of ENaC activity in the CCD indicates that the inhibitory effect of AA is mediated by CYP-epoxygenase AA metabolites. Second, no significant CYP-epoxygenase activity can be detected in oocytes and cultured cell lines (unpublished data). Thus, it is possible that AA at physiological concentrations inhibits ENaC via a CYP-epoxygenase–dependent pathway, whereas at high concentrations it decreases the ENaC activity by AA-mediated endocytosis. Also, it is very likely that ETYA at high concentrations or for a prolonged incubation may inhibit ENaC by a mechanism other than EET.

The observation that ETYA cannot mimic the effect of AA suggests that the effect of AA is mediated by AA metabolites. The major enzymes responsible for AA metabolism in the kidney include COX, lipoxygenase, and CYP monooxygenase (Roman, 2004). Moreover, a large body of evidence indicates that both COX-dependent and CYP enzyme–dependent metabolites of AA play an important role in the regulation of membrane transport in the kidney (Escalante et al., 1991; Ma et al., 1994; Wang et al., 1996; Roman, 2004). The main metabolites of a COX-dependent pathway of AA are prostaglandins such as PGE2, which have been shown to inhibit the transepithelial Cl transport (Culpepper and Andreoli, 1983), bicarbonate transport (Good, 1996), apical 70 pS K channels in the TAL (Liu et al., 2000), and Na transport in the rabbit collecting tubule (Stokes and Kokko, 1977). Moreover, PGE2 has been shown to suppress the vasopressin-induced increase in water channels (Hébert et al., 1990; Hebert et al., 1991). However, the observation that inhibition of COX did not abolish the AA-induced inhibition of ENaC excluded the role of the COX-dependent AA metabolite in mediating the effect of AA. Also, it is unlikely that 11,12-EET–induced inhibition of ENaC required the involvement of COX because 11,12-EET cannot be metabolized by COX.

AA can be metabolized by CYP-dependent ω-hydroxylases and the main products are HETE derivatives such as 19- or 20-HETE (Roman, 2004). 20-HETE has been demonstrated to inhibit Na/Cl/K cotransporter and the apical 70 pS K channel in the TAL (Escalante et al., 1991; Wang and Lu, 1995). However, it is unlikely that the effect of AA is mediated by the CYP-dependent ω-hydroxylases because DDMS did not block the effect of AA on ENaC. Also, we observed that application of 20-HETE had no effect on ENaC.

Four lines of evidence indicate that the effect of AA is mediated by 11,12-EET: (1) inhibition of CYP-epoxygenase abolishes the effect of AA on ENaC; (2) addition of 11,12-EET, but not other EETs, mimics the effect of AA and inhibited ENaC; (3) 11,12-EET blocks the ENaC in the presence of a CYP-epoxygenase inhibitor;

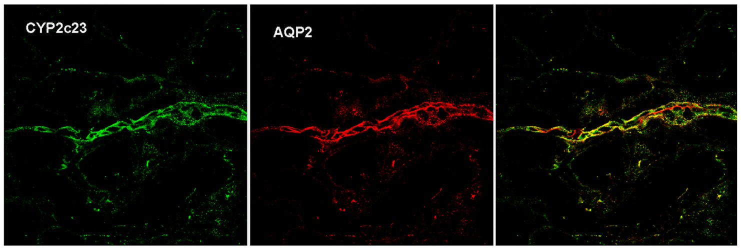

Figure 12. Confocal images showing the CYP2C23 (green) and AQP2 (red) staining in the renal cortex. The bar represents 10 μM.

(4) 11,12-EET is present in the CCD. The role of EET in the regulation of ion channels is well established. EET has been shown to inhibit cardiac L-type Ca^{2+} channels (Chen et al., 1999a) and activate the Ca^{2+}-dependent large conductance K channels in smooth muscle cells of renal vessels (Zou et al., 1996).

Although several CYP epoxygenases, such as CYP2C11, 2C12, 2C23, and 2C24, have been shown to be expressed in the renal tubules and are capable of converting AA to EET (Roman, 2004), the CYP2C23 family has been considered to be the major isoform in the kidney to generate EET. Our unpublished observation has indicated that CYP2C23 is expressed in the CCD. Moreover, 11,12-EET accounts for over 60% of the total renal EET (Holla et al., 1999). EET has been shown to regulate Na transport in the CCD. For instance, 5,6-EET inhibits the Na/H exchanger via a Ca^{2+}-dependent pathway in the rabbit CCD (Sakairi et al., 1995). However, the effect of 5,6-EET on the Na/H exchanger in the CCD is mediated by a COX-dependent pathway because indomethacin abolished the effect of 5,6-EET.

The mechanism by which 11,12-EET inhibits ENaC is not clear. We were not able to test the effect of either AA or 11,12-EET on ENaC in inside-out patches because the channel activity ran down progressively. Therefore, it is not known whether the effect of 11,12-EET on ENaC results from direct blockade of ENaC or is due to modulating protein kinases or phosphatases. ENaC has been shown to be stimulated by PKA and PTK and inhibited by PKC (Marunaka and Eaton, 1991; Ling et al., 1992; Matsumoto et al., 1993; Frindt and Palmer, 1996; Frindt et al., 1996). On the other hand, a large body of evidence has demonstrated that AA and EET modulate a variety of signal transduction pathways (Buckley and Whorton, 1995; Firsov et al., 1995; Chen et al., 1999b; Spector et al., 2003; Cheng et al., 2004). It has been reported that AA inhibits cAMP formation induced by vasopressin in the TAL (Firsov et al., 1995), whereas it stimulates tyrosine phosphorylation (Buckley and Whorton, 1995). EET has been shown to diminish the effect of vasopressin on water permeability in the CCD, presumably by decreasing cAMP formation (Hirt et al., 1989). Also, 14,15-EET has been demonstrated to activate PTK-dependent signaling (Chen et al., 1999b). Thus, it is possible that the effect of EET on ENaC may be partially mediated by regulation of kinase activity. Further experiments are required to test this hypothesis.

The physiological importance of the present finding is that 11.12-EET may play an important role in the regulation of renal Na transport. The rate limiting step of transepithelial Na transport in the CCD is the apical Na conductance (Koeppen and Stanton, 1992), which is determined by the number of Na channels in the apical membrane and the channel open probability. Increases in either Na channel numbers or channel open probability should augment the apical Na conductance and lead to stimulation of Na absorption. For instance, Liddle's syndrome, an inherited form of hypertension, is the result of decreasing endocytosis of ENaC and accordingly increasing the apical ENaC numbers in the CCD (Shimkets et al., 1994; Snyder et al., 1995). Although two types of Na channels, 4-6 pS and 28 pS, have been identified in the collecting duct (Palmer and Frindt, 1986; Light et al., 1988), it is generally accepted that the 4-6 pS ENaC is mainly responsible for the Na entry across the apical membrane in the CCD (Garty and Palmer, 1997). Therefore, the present finding suggests that AA and its metabolites play a role in the regulation of apical Na conductance and Na transport in the CCD.

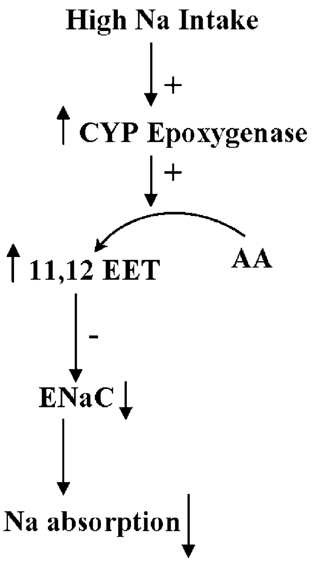

FIGURE 13. A schema illustrating the mechanism by which high Na intake suppresses ENaC activity.

Aldosterone is the main hormone regulating the ENaC activity; a high plasma aldosterone increases, whereas low aldosterone decreases, the apical ENaC activity (Koeppen and Stanton, 1992). Moreover, the early effect of aldosterone is to increase the ENaC open probability, whereas the late effect is produced by increasing the ENaC number in the apical membrane of the CCD. In addition, Na diet also plays an important role in the regulation of ENaC activity; low Na intake increases the apical ENaC number (Palmer et al., 1994) and the effect of low Na on ENaC is mediated by aldosterone. High Na intake decreases the apical ENaC number and the effect of high Na intake on ENaC activity is generally considered to be the result of decreasing aldosterone levels. However, the observation that high Na intake significantly increases renal EET formation suggests that EET may also be responsible for decreasing ENaC activity induced by high Na intake. In this regard, it has been shown that inhibition of CYP-epoxygenase AA metabolism causes hypertension in rats on a high Na diet and that removal of an epoxygenase inhibitor decreases the blood pressure in rats maintained on a high Na diet (Makita et al., 1994). Fig. 13 is a schema illustrating the possible role of 11,12-EET in the regulation of Na transport in the CCD. We speculate that a high Na intake stimulates the activity of CYP-epoxygenase and increases the 11,12-EET levels, which in turn inhibit ENaC and suppress Na transport in the CCD. We conclude that AA inhibits ENaC activity via CYP-epoxygenase AA metabolic pathway and that 11,12-EET mediates the inhibitory effect of AA on ENaC in the CCD.

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