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Effects of Lipids on ENaC Activity in Cultured Mouse Cortical Collecting Duct Cells

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Abstract Direct effects on epithelial Na^+ channels (ENaC) activity by lipids, e.g., arachidonic acid (AA), eicosatetraynoic acid (ETYA), linoleic acid (LA), stearic acid (SA), hydroxyeicosatetraenoic acid (HETE), 11,12–epoxyeicosatrienoic acid (EET), (PGF2), and (PGE2), in cultured mouse cortical collecting duct (M1) cells were clarified by using single-channel recordings in this study. In a cell-attached recording, a bath application of 10 μM AA significantly reduced the ENaC open probability (NPo), whereas 10 μM ETYA or 5 μM LA only induced a slight inhibition. The inside-out recording as a standard protocol was thereafter performed to examine effects of these lipids on ENaC activity. Within 10 min after the formation of the inside-out configuration, the NPo of ENaC in cultured mouse cortical collecting duct (M1) cells remained relatively constant. Application of ETYA or LA or SA exhibited a similar inhibition on the channel NPo when applied to the extracellular side, suggesting that fatty acids could exert a nonspecific inhibition on ENaC activity. 11,12-EET, a metabolite of AA via the cytochrome P450 epoxygenase pathway, significantly inhibited the ENaC NPo, whereas 20-HETE, a metabolite of AA via the hydroxylase pathway, only caused a small inhibition of the ENaC NPo, to a similar degree as that seen with ETYA and LA. However, both PGE2 and PGF2α significantly enhanced the ENaC NPo. These results suggest that fatty acids exert a nonspecific effect on ENaC activity due to the interaction between the channel proximity and the lipid. The opposite effects of 11,12-EET and prostaglandin (PG) implicate different mechanisms in regulation of ENaC activity by activation of epoxygenase and cyclooxygenase.

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Introduction

Epithelial Na^+ channels (ENaC) expressed on the apical membrane of kidney distal and collecting ducts are responsible for hormone-regulated Na^+ reabsorption. Two basic steps compose this Na^+ reabsorption: Na^+ enters into the cells via ENaC on the apical membrane following the Na^+ gradient, and it is then extruded to the interstitial fluid by basolateral Na^+–K^+-ATPase. The amount of Na^+ uptake will be determined by the number of channels (channel density) and channel activity. Although the direct activation mechanism of ENaC remains unclear, protein kinase A, the small G protein K-Ras (Eaton et al. 2001; Staruschenko et al. 2004a, b), serum glucocorticoid-inducible kinase (Chen et al. 1999; Friedrich et al. 2003), and the channel-activated protease (CAP1) (Vallet et al. 1997) have been demonstrated to increase ENaC activity, whereas protein kinase C, $[Na^+]_i$ (Anantharam et al. 2006), arachidonic acid (AA) (Worrell et al. 2001), the products of cytochrome P450 (CYP), e.g., 11,12–epoxyeicosatrienoic acid (EET) (Wei et al. 2004), and the reduction of phosphatidylinositol biphosphate (PIP2) (Ma et al. 2002; Yue et al. 2002) have been shown to decrease ENaC activities.

AA is found in the *sn*-2 position of membrane phospholipids and can be liberated primarily by phospholipase (PLA). Upon release, AA may initiate signaling or can be metabolized into a wide range of products via cyclooxygenases (COX), lipoxygenase, and CYP. AA and its metabolites act in a diverse range of physiological and pathological roles in water and Na^+ homeostasis in the kidney (Breyer and Breyer 2000; Breyer et al., 1996,

1998). In addition to interactions with specific receptors (Breyer et al. 2000), lipids can also directly bind to ion channels to alter their activities, resulting in either inhibition (Oliver et al. 2004) or stimulation (Chyb et al. 1999).

By using transepithelial electric resistance methods, a previous study has shown that the decrease of apical AA by a cPLA antagonist significantly increases transepithelial Na^+ current whereas the decrease of basolateral AA reduces Na^+ transport, suggesting that AA can lead to inhibition of ENaC activity (Worrell et al. 2001). In the oocyte expression system, AA, as well as its analogue, eicosatetraynoic acid (ETYA), moderately decreases ENaC whole cell currents by inhibiting ENaC exocytosis and increasing ENaC endocytosis (Carattino et al. 2003). However, AA fails to reduce ENaC activity when CYP-epoxygenase is blocked, suggesting that 11,12-EET as a metabolite of AA inhibits ENaC activity (Wei et al. 2004). It still remains unclear whether ENaC activity, including conductance and open probability, is altered by lipids regardless of the channel density and other second messengers. To prevent the interference of second messengers, single-channel recording was used in this study to clarify the direct effects of the lipids on ENaC channels.

Materials and Methods

Cell Culture

M1 cells (mouse kidney cortical collecting duct cells) were purchased from the European Collection of Cell Cultures at the 21st passage. Cells were grown in a medium containing DMEM:Ham's F12 medium (1:1) (Sigma), 2 mM glutamine (Gibco), 5 μM dexamethasone (Sigma), and 5% fetal bovine serum (Sigma) in a 5% CO_2 and 37°C incubator. Aldosterone (1.5 μM) (Sigma) was added into the culture medium 24 h before the experiments to stimulate cells. When cells reached 70% confluence, they were seeded in a low density to either the coverslip or the culture inserts (BD).

Single-Channel Patch Clamp Recording

Single-channel recordings were performed as previously described (Gorelik et al. 2002). Briefly, a coverslip or insert containing grown M1 cells was transferred into a recording chamber mounted on a Nikon inverted microscope (Nikon TE 2000U). A patch pipette with a resistance of 7 MΩ was fabricated from a borosilicate glass capillary (1.5 o.d., 0.86 i.d.) (Warner) on a Sutter Puller (P97). Bath solutions contained the following (in mM): 110 NaCl, 4.5 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 5 HEPES, 5 Na-HEPES, pH 7.2. Pipette solution contained the following (in mM): 110 NaCl, 4.5 KCl, 0.1 EGTA, 5 HEPES, 5 Na-HEPES, pH 7.2. Currents were recorded with an Axon 1D amplifier and Axon clampex 9.0.

The data were acquired at 20 kHz and filtered by a 1-kHz low-pass filter. The channel events were analyzed by pclampfit 9.0 (single-channel search in analyze function). Data were further filtered at 200 Hz before data analysis. A 50% threshold cross method was used to determine valid channel openings. When multiple channel events were observed in each patch, the total number of functional channels (N) in the patch was determined by observing the number of peaks detected on all point amplitude histograms. NPo (the product of the number of channels and the open probability) or the open probability (Po) itself was used to measure the channel activity within a patch. The NPo was calculated as previously described (Yue et al. 2002). Because the recording membrane patch usually contained multiple channels, in most cases, the changes in the NPo (not Po) were directly observed and compared. As a result of the variance of the channel open probability, 2–5 min of single-channel recording before lipid application was normally used as the control. The open probability of the channel during the application of lipids (whole duration excluding the noise) was directly compared with that of the control. This ratio was used to determine the effects of lipids on ENaC activity. In some cases, the NPo of ENaC during the application of lipids was compared with that of ENaC in medium after the lipids were washed away. The data were used to confirm the observations. The data are presented as mean ± standard error of the mean, and statistical differences were compared by Student's paired *t*-test, taking $P < 0.05$ as significant.

There is an inevitable fluid turbulence during bath medium exchange, for example during valve opening and closing, and this will cause a transient noise that is composed of an initial spike and a baseline sine wave. The noise component of the currents was excluded from the NPo analysis. Control experiments have been performed by switching perfusion with normal bath medium, and the NPo of the currents was not significant altered.

Chemicals

The following chemicals were used: AA (Alexis), linoleic acid (LA) (Alexis), ETYA (Alexis), stearic acid (Sigma), 11,12-EET (Biomol), hydroxyeicosatetraenoic acid (20-HETE) (Biomol), N-methylsulfonyl-6-(2-propargyloxyphenyl) hexanamide (PPOH) (Cayman), N-methylsulfonyl-12,12-dibromododec-11-enanide (DDMS) (Cayman), AH6809 (Biomol), SC-51322 (Biomol), PGE2 (Biomol), and PGF2α (Biomol). Most chemicals were dissolved in ethanol and made up as 1000 to 5000 times stock. All recording solutions were made on the day of experiments. The solvent at the same dilution was tested alone in controls and had no effect on the results.

Results

Functional ENaC Channels Expressed in M1 Cells

Single-channel recording was used to characterize the ENaC channels in M1 cells. In a cell-attached recording, an inward current was detected when the pipette voltage was held at +20 mV and +60 mV. These inward currents possessed a slope conductance of 5.1 ± 0.25 pS (n = 15) between the command voltages (20 and 60 mV, hyperpolarization). The cell membrane under a patching pipette was excised from the cell to form either the inside-out or the outside-out recording configuration. In an outside-out recording, the currents with a slope conductance of 5.15 ± 0.15 pS (n = 15) were shown in Fig. 1a. A bath application of 0.5 μM amiloride significantly reduced the channel open probability, shown as the currents flickered and the major open state stepped toward the closing level. A high concentration of amiloride (5 μM) then almost abolished this current (Fig. 1a). Currents with the same conductance were also observed in the inside-out recordings (n = 60) (Fig. 1b). The summary of the excised membrane recordings was plotted as shown in Fig. 1c. The data above suggested that these currents were carried out by ENaC channels. In addition, previous reports have also shown that the rundown of ENaC currents in the inside-out recording are due to the lack of PIP2 (Yue et al. 2002). In M1 cells, significant current rundown of ENaC was not normally observed within 10 min of the formation of the inside-out recording configuration (Fig. 2d). The selected NPo values of ENaC in M1 cells (seven individual cells), which cover the range of NPo values observed in M1 cells, are presented in Fig. 2d.

Effects of AA and Other Fatty Acids on ENaC Activity from the Extracellular Side

Within 10 min, the bath application of AA at 10 μM significantly reduced the ENaC NPo to 0.08 ± 0.05% of the control (n = 5), as shown in Fig. 2a ($P < 0.005$), while the cell-attached recording was performed. Incubating cells with AA for a longer period did not enhance the inhibition effect, as described previously (Carattino et al. 2003). ETYA, an analogue of AA but resistant to AA degenerative

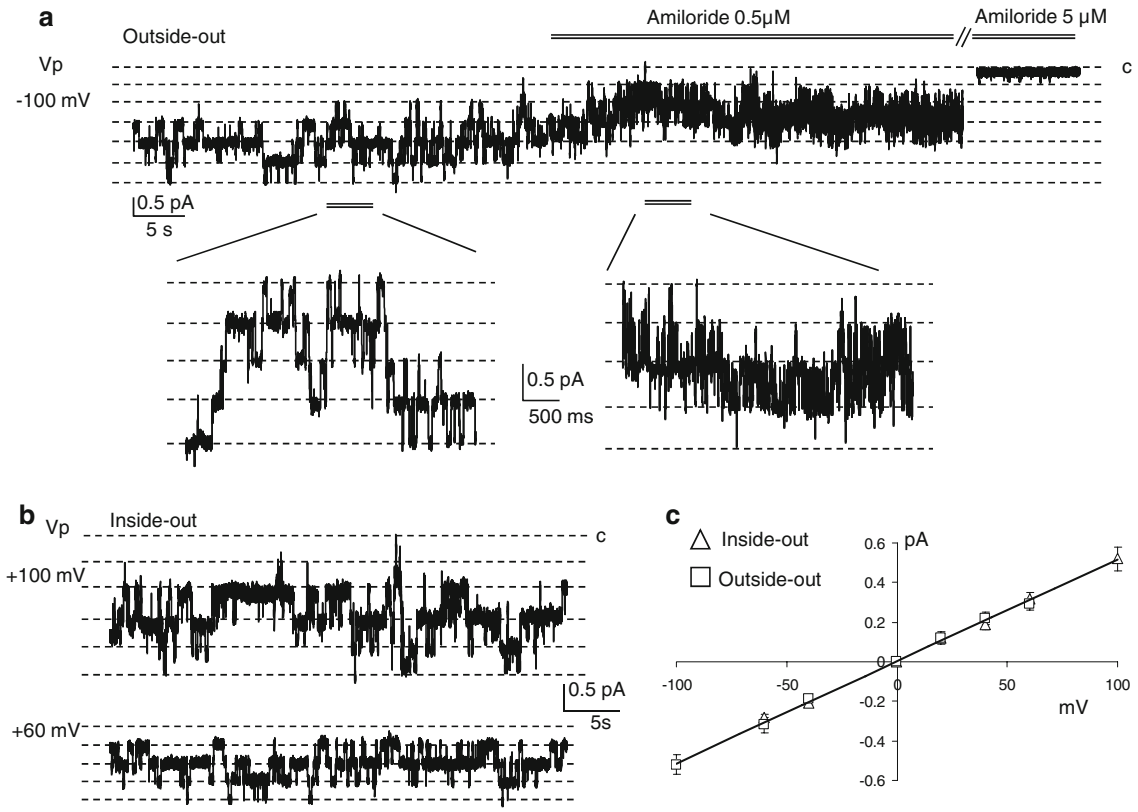

Fig. 1 Single-channel currents of ENaC in M1 cells. **a** In an outside-out recording, the currents were monitored when the pipette voltage was held at −100 mV. The bath application of 0.5 μM amiloride significantly reduced the ENaC open probability, and 5 μM amiloride almost abolished the ENaC currents. **b** In an inside-out recording, single-channel currents were detected when the pipette voltage was held at +100 mV and +60 mV. **c** Currents corresponding to the voltages from inside-out (n = 60) and outside-out recordings (n = 15) were plotted. The points were fitted by a line ($\gamma^2 = 0.99$)

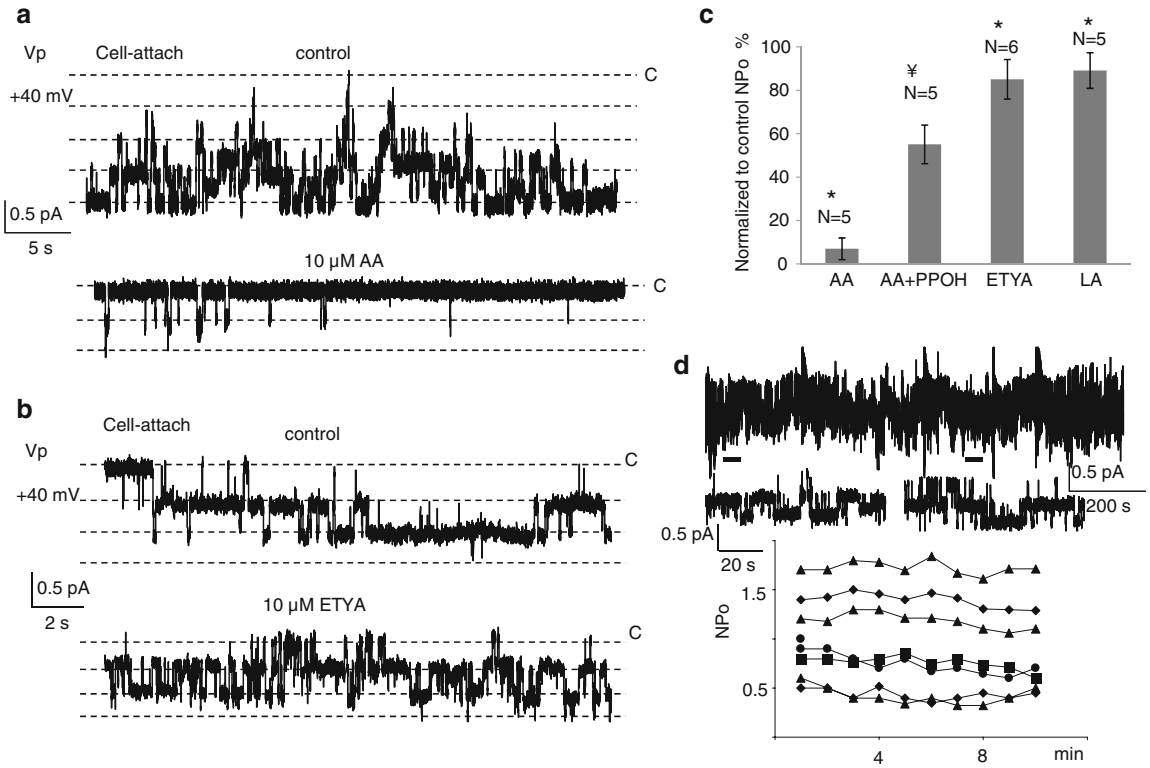

Fig. 2 Effects of PUFA on ENaC activity in cell-attached recordings. **a** In a cell-attached recording, the bath application of 10 μM AA significantly reduced the ENaC NPo. **b** In a cell-attached recording, the bath application of 10 μM ETYA slightly reduced the ENaC NPo. **c** The bar shows the effects of extracellular PUFA on ENaC NPo. **d** A trace of single-channel current from an inside-out recording. Summary plots of the NPo from seven individual cells 10 min after the formation of the inside-out configuration

enzymes, was used to determine whether the inhibitory effect of AA is a result of the metabolites of AA. The NPo of the ENaC channel was slightly reduced to 85.1 ± 9.1% (n = 6) of the control by ETYA (Fig. 2b) ($P < 0.05$). In addition, extracellular application of LA also reduced the NPo of ENaC to 89.1 ± 8.2% (n = 5) of the control (Fig. 2c) ($P < 0.05$). In an outside-out recording, a bath application of AA reduced the NPo of ENaC to 77.5 ± 6.1% of the control (n = 5). These results indicate that the lipid can alter the membrane's physical properties, resulting in a slight change in channel kinetics. However, the major inhibition effect of AA on ENaC is accounted for by the metabolites of AA but not via a nonspecific effect of the lipid.

Effects of AA and Other Lipids on ENaC Activity from the Cytoplasmic Side

In most of the inside-out recordings, cells were held by a pipette voltage of either 60 mV or 100 mV. In the inside-out recordings, lipids can directly interact with the cytoplasmic membrane and the effects of lipids on ENaC should be instantaneous. Therefore, our standard protocol was to incubate the patch of the membrane with the lipids for up to 5 min. Some experiments with longer incubation times, exceeding 9 min, were also performed and no further effects on ENaC activity due to the lipids were observed. Application of 10 μM AA only induced a small reduction of the ENaC NPo, to 86.1 ± 4.2% (n = 10) of the control (Fig. 3a) ($P < 0.05$). Similar effects were observed by application of ETYA (n = 9), LA (n = 8), or stearic acid (n = 6) on the cytoplasmic membrane (Fig. 3b). These observations suggested that a significant inhibition effect of AA on the ENaC NPo resulted from the AA metabolites, but not from AA itself.

Inhibition Effect of 11,12-EET on ENaC Channels

11,12-EET as a metabolite of AA via the epoxygenase pathway exerted a significant inhibition on the ENaC NPo when it was applied directly to the cytoplasmic membrane. In an inside-out recording, the NPo of ENaC was reduced to 5.3 ± 2.1% (n = 12) of the control by a bath application of 250 nM of 11,12-EET and 7.4 ± 2.5% (n = 11) of the control by 100 nM of 11,12-EET (Fig. 4). The other products, e.g., 20-HETE, as metabolites of AA via the CYP-dependent ω-hydroxylation pathway, slightly altered the NPo of ENaC, as did ETYA (data not shown).

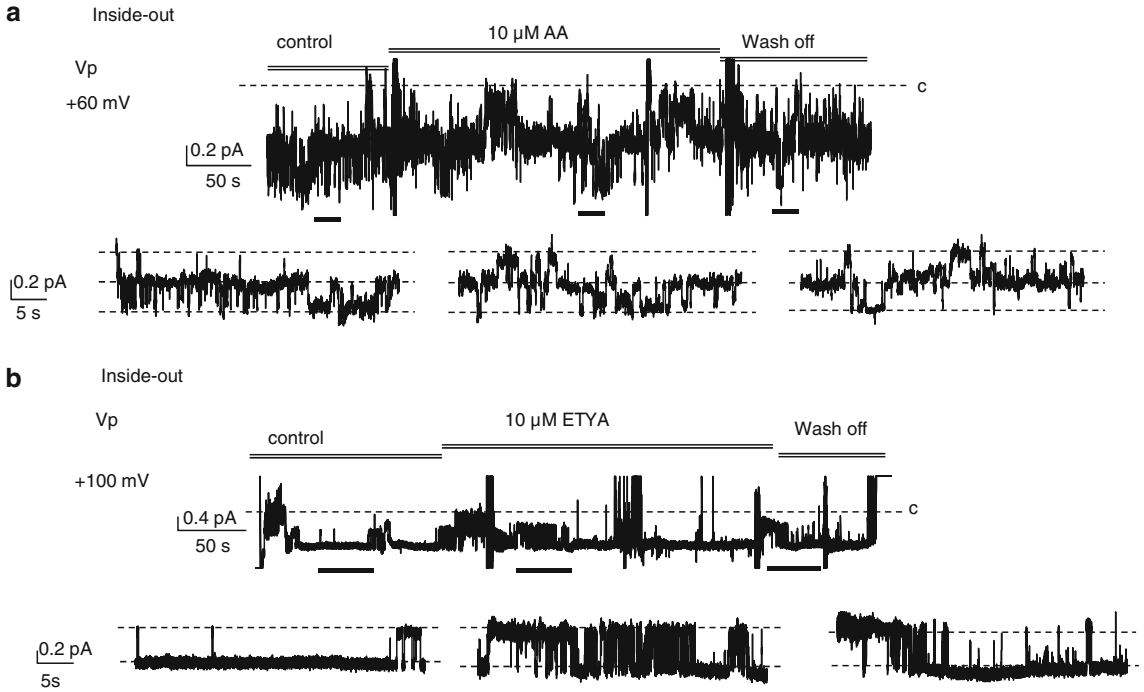

Fig. 3 Effects of AA and ETYA on ENaC activity in inside-out recordings. **a** In an inside-out recording, the bath application of 10 μM AA slightly reduced the ENaC open probability, and this effect of AA was reversed when AA was washed away. **b** In an inside-out recording, the bath application of 10 μM ETYA slightly reduced the ENaC open probability, and this effect was reversed when ETYA was washed away

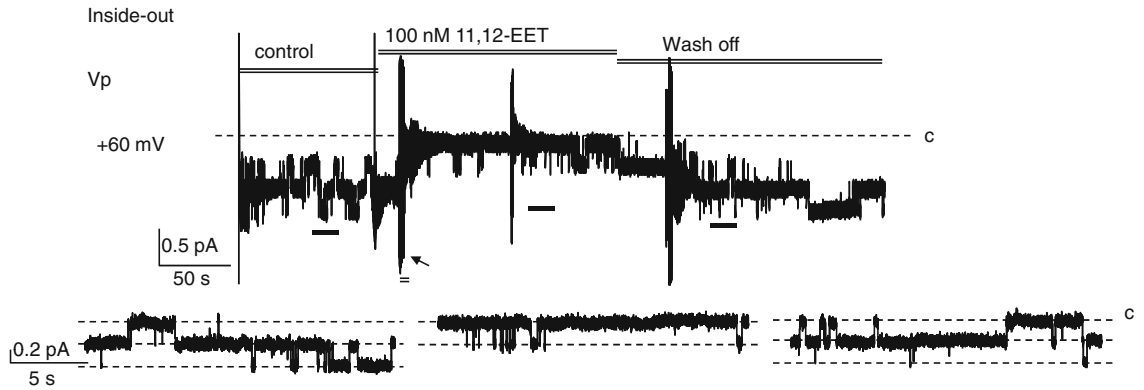

Fig. 4 Effects of 11,12-EET from the cytoplasmic side on ENaC activity. In an inside-out recording, 11,12-EET at 100 nM significantly reduced the ENaC open probabilities. This effect was reversed when the 11,12-EET was washed away. The *arrow* indicates the noise induced by the fluid turbulence in the recording bath

In the outside-out recordings, a bath application of 250 nM of 11,12-EET inhibited ENaC activity to 8.3 ± 3.1% (n = 5) of the control, which is similar to the results obtained from cytoplasmic application of 11,12-EET ($P > 0.05$). In a cell-attached recording, a bath application of 10 μM AA reduced the NPo of ENaC to 55.1 ± 8.9% (n = 5) of the control when cells were preincubated with PPOH (15 μM), which is significantly different from AA effects on cells without treatment ($P < 0.05$) (Fig 2c).

Effects of Prostaglandin E (PGE) and Prostaglandin F (PGF) on ENaC Activity

PGF2 and PGE, the major metabolites of AA via the COX pathway, are abundant in CCD cells. They are generated in the cellular plasma and diffuse out of the cell. In an inside-out recording, the application of PGE enhanced the NPo of ENaC to 135.1 ± 14.2% (n = 12) (Fig. 5a) of the control ($P < 0.05$) and addition of PGF2 to the cytoplasmic membrane enhanced the NPo of ENaC to 167.3 ± 15.1%

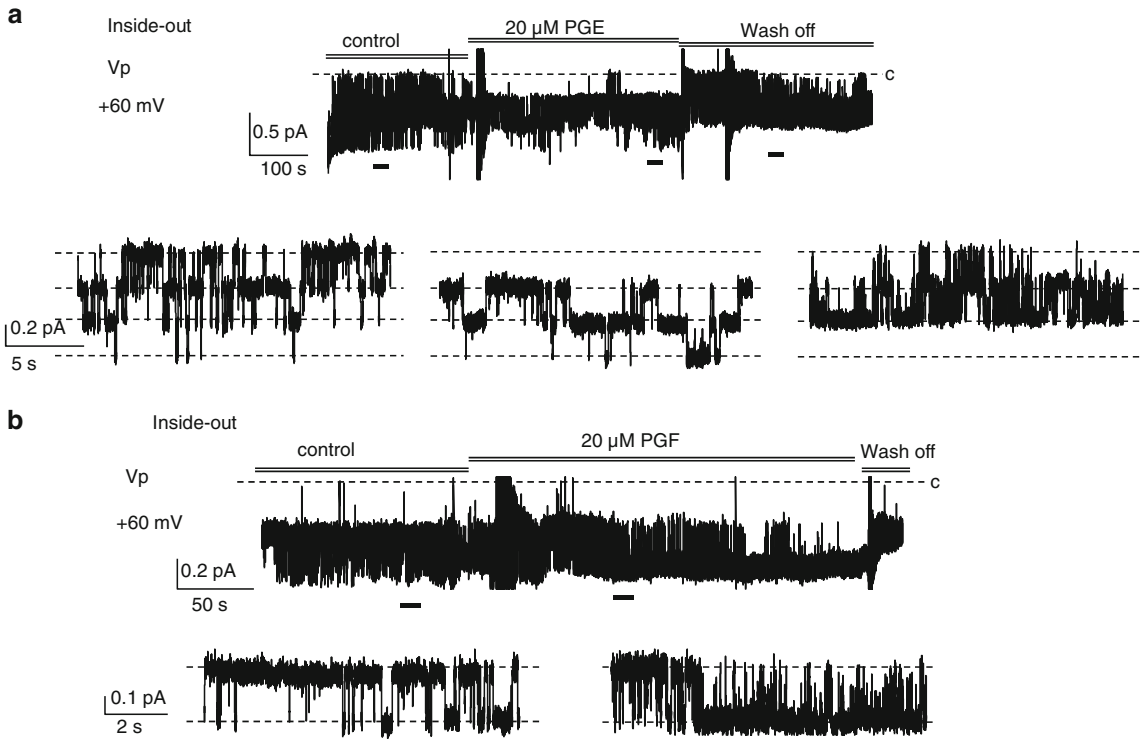

Fig. 5 Effects of PGE2 and PGF2α from the cytoplasmic side on ENaC activity. **a** In an inside-out recording, the bath application of 20 μM PGE2 enhanced the ENaC open probability. **b** In an inside-out recording, 20 μM PGF2α slightly enhanced the ENaC open probability. This effect was reversed when PGE2 was washed away

(n = 11) (Fig. 5b) of the control ($P < 0.05$). Changes in the conductance of the ENaC channels were not observed with the application of either PGF2 or PGE. In addition, PGE2, at a physiological concentration of 2 μM (Els and Helman 1997), also enhanced the NPo of ENaC to 153.1 ± 111.3% (n = 4). Furthermore, E-prostanoid receptor antagonists, e.g., SC-51322 (2 μM) and AH6809 (5 μM), have been included in the pipette during some inside-out recording (n = 20), but no significant effects were observed on the augmented effects of prostaglandin (PG) on the ENaC NPo.

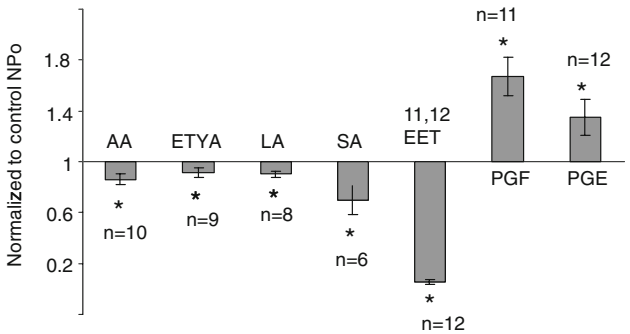

Fig. 6 Effects of lipids from the cytoplasmic side on ENaC activities. This figure shows the analyzed results about the effects of the lipids on ENaC NPo. Data were normalized to control

In conclusion, cytoplasmic AA, ETYA, LA, and stearic acid (SA) slightly reduced the NPo of ENaC, whereas 11,12-EET almost abolished ENaC activity. Cytoplasmic PGE and PGF both significantly enhanced the NPo of ENaC (Fig. 6).

Discussion

The data presented here demonstrate that AA inhibits ENaC activity in M1 cells. This inhibition effect is mediated by 11,12-EET, a metabolite of AA via CYP epoxygenase, but not via AA per se. The unsaturated fatty acids from either the extracellular or intercellular side of the cell membranes slightly reduced the ENaC channel open probability, whereas both PGF2 and PGE, the major metabolites of AA via COX, significantly enhanced the ENaC channel open probability. Because the lipids and their metabolites are constantly present in the cellular cytoplasm, their direct interaction with ENaC channels might serve as a mechanism in the regulation of ENaC activities and subsequently determine Na^+ reabsorption.

The previous studies have shown that ETYA, at high concentrations of 40 μM (Worrell et al. 2001) and 50 μM (Carattino et al. 2003), inhibits ENaC activity in *Xenopus* oocytes. Because of the similar effects of ETYA and AA, AA was considered a direct regulator of ENaC.

Controversially, ETYA has also been shown to exert no effect on ENaC activity from rat CCD cells (Wei et al. 2004), resulting in a conclusion that metabolites of AA, rather than AA itself, inhibit ENaC activity. We found that there is a small inhibition of ENaC activity caused by ETYA, and this small inhibition is due to nonspecific effects of fatty acids. Application of LA, ETYA, and stearic acid from either the extracellular or intracellular side exerts similar slight inhibitions of ENaC. However, differences in AA's inhibitory effect on ENaC by cytoplasmic application revealed that metabolites of AA, rather than AA itself, primarily inhibit ENaC. This is consistent with previous work (11,12-EET) (Wei et al. 2004).

In the toad bladder, an increase of Na^+ reabsorption is associated with an increased turnover of phospholipids (Goodman et al. 1975), which implicates the involvement of PLA (Yorio and Bentley 1978). Inhibition of PLA2 by aristolochic acid significantly increases the Na^+ conductance in A6 cells, suggesting that cPLA2 is tonically active in A6 cells (Worrell et al. 2001). The cPLA2 constantly liberates free AA from the *sn*-2 position of the membrane lipids. The free AA is the substrate for a number of enzymes, e.g., COX, CYP epoxygenase, and CYP hydroxylase, and it can degenerate to a number of metabolites, e.g., PGE2, PGF2, EET, and HETE. Either free AA or its metabolites on the cytoplasmic side may act as signaling molecules to directly regulate ion channels. Previous studies have demonstrated that polyunsaturated fatty acids including AA and LA could directly mediate the activities of ion channels including transient receptor potential (TRP) (Chyb et al. 1999), big conductance Ca^2-activated K channel (BK) (Denson et al. 2000), K^+ channels (Kim and Clapham 1989; Oliver et al. 2004; Ordway et al. 1989), and this regulation is not due to alterations in the membrane fluidity by PUFA. In this study, we found that fatty acids, including saturated fatty acids, could slightly inhibit ENaC activities in a nonspecific way. Unlike other channels, ENaC possesses only two transmembrane domains and cytoplasmic C- and N-terminals. Other evidence suggested that ENaC may associate with the cholesterol enriched microdomains (Hill et al. 2002), which are detergent insoluble. The fatty acids may interact with either a subunit of the ENaC channel (Carattino et al. 2003), or these lipids may form rafts to alter the physical proximity, resulting in changes in channel activities.

Prostaglandins comprise a diverse family including PGE2, PGF2α, PGD2, PGI2, and thromboxane A2 (TXA2) (Breyer and Breyer 2001; Breyer et al. 2002; Sugimoto and Narumiya 2007). These prostanoids are abundantly produced in the kidney (Bonvalet et al. 1987; Farman et al. 1986; Hebert et al. 2005; Smith 1992; Smith et al. 1991), where they act locally via eight specific transmembrane G protein–coupled receptors designated EP 1–4 (for E-prostanoid receptor), FP, DP, IP, and TP (Breyer et al. 2002; Nusing and Seyberth 2004; Sugimoto 2006; Wu and Liou 2005), respectively. PGE2 is the major prostaglandin produced along the collecting duct (Hebert et al. 1995), where it potently regulates solute and water transport (Breyer and Breyer 2000; Breyer et al. 1996). It is well established that PGE2 exerts its effects by either stimulating or inhibiting salt and water reabsorption through its interaction with the cell surface EP receptors in an autocrine or paracrine fashion (Narumiya 1994). The controversial effects are generally considered to be due to the diverse signaling pathways initiated by distinct EP receptors (Guan et al. 1998). However, as we demonstrated in this report, PGs can also directly affect ENaC without EP receptors because they lack a cellular mediator, e.g., Ca^{2+} or cAMP, to cope with the activation of G protein-coupled receptors in the inside-out recordings. Cytoplasmic prostaglandins directly enhance the NPo of ENaC. Because of their membrane permeability, luminal PGs might also stimulate ENaC after they enter into the cytoplasm. Many observations may possibly account for this direct activation. For example, infusion PGs in renal filtrates increases Na^+ reabsorption (Breyer and Harris 2001; Hornych et al. 1975; Lazzeri et al. 1995). PGE2 augments amiloride-sensitive Na^+ transport in frog skin (Kokko et al. 1994; Matsumoto et al. 1997) and in MDCK (Wegmann and Nusing 2003) and A6 cells (Nielsen 1990).

Our results are in disagreement with the observations of Els and Helman (1997), which found that PGE2 decreases the NPo of Na^+ channels by unknown mechanisms. Several explanations of this discrepancy are possible. Most obviously, there could be a species difference because frog skin was investigated in the previous study, whereas our study was performed on mouse kidney cells. Second, there could be time-dependent differences. In the tissue study of frog skin, changes in short circuit currents happen over minutes. In patch clamping experiments, the exposure of the inner surface of the membrane is fairly brief. Effects of PG on ENaC are instantaneous and occur within seconds. Finally, the interference of second messengers should be seriously considered when the study is performed on a tissue level. Within minutes, application of PGE could stimulate a variety of signaling pathways via EP receptors (Breyer and Breyer 2001). Results would therefore be profound. In this study, we provided a simple and clear conclusion that excludes effects of second messengers.

Consistent with the previous studies, 11,12-EET, but not HETE, plays a primary role in inhibiting ENaC activity. This effect is due to the direct interaction of 11,12-EET with ENaC channels, although the interaction site remains unclear. Accumulated evidence has suggested that 11,12-EET is the major signaling molecule in inhibiting ENaC activity (Sun et al. 2006; Wei et al. 2004, 2006). The expression of CYP epoxygenase, which mediates the

product of 11,12-EET from AA, could be regulated by the diet of Na^+ uptake (Sun et al. 2006). It partly explains the observation that removal of an epoxygenase inhibitor decreases the blood pressure in rats maintained on a high Na^+ diet (Makita et al. 1994). However, some studies reported there is either lack of epoxygenase or deficient of epoxygenase activity in cultured cells (Michaelis et al. 2005). Our results in this study confirm that 11,12-EET strongly inhibits ENaC by direct interaction.

There are opposite regulations in ENaC channel activity by different AA metabolites. Products of epoxygenase inhibit ENaC channels whereas products of COX augment ENaC activity. According to our experiments, lipids are strongly suggested to directly interact with the ENaC channels via the binding sites in the ENaC molecular structure. The defined ENaC activity will be determined by lipid affinity to ENaC channels and spatial distribution of different AA metabolism enzymes. In A6 cells, application of aristolochic acid from the apical membrane increases transepithelial Na^+ current, whereas application from the basolateral side reduces Na^+ transport (Worrell et al. 2001). Basolateral application of ibuprofen mimics the effects of inhibition of cPLA via basolateral application of aristolochic acid, suggesting that the distribution of COX in A6 is likely close to the basolateral membrane.

This study provides direct evidence to reveal the direct effects of common cytoplasmic lipids on ENaC activities because membrane patches in inside-out recordings containing channels are physically isolated from the rest of the cell and cytoplasmic factors are absent. This evidence will be help us better understand the regulation mechanisms of ENaC by lipids, although many issues remain to be resolved.

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References

Anantharam A, Tian Y, Palmer LG (2006) Open probability of the epithelial sodium channel is regulated by intracellular sodium. J Physiol 574:333–347

Bonvalet JP, Pradelles P, Farman N (1987) Segmental synthesis and actions of prostaglandins along the nephron. Am J Physiol 253:F377–F387

Breyer MD, Breyer RM (2000) Prostaglandin receptors: their role in regulating renal function. Curr Opin Nephrol Hypertens 9:23–29

Breyer MD, Breyer RM (2001) G protein-coupled prostanoid receptors and the kidney. Annu Rev Physiol 63:579–605

Breyer MD, Harris RC (2001) Cyclooxygenase 2 and the kidney. Curr Opin Nephrol Hypertens 10:89–98

Breyer MD, Jacobson HR, Breyer RM (1996) Functional and molecular aspects of renal prostaglandin receptors. J Am Soc Nephrol 7:8–17

Breyer MD, Zhang Y, Guan YF, Hao CM, Hebert RL, Breyer RM (1998) Regulation of renal function by prostaglandin E receptors. Kidney Int Suppl 67:S88–S94

Breyer RM, Kennedy CR, Zhang Y, Breyer MD (2000) Structure-function analyses of eicosanoid receptors. Physiologic and therapeutic implications. Ann N Y Acad Sci 905:221–231

Breyer RM, Kennedy CR, Zhang Y, Guan Y, Breyer MD (2002) Targeted gene disruption of the prostaglandin E2 EP2 receptor. Adv Exp Med Biol 507:321–326

Carattino MD, Hill WG, Kleyman TR (2003) Arachidonic acid regulates surface expression of epithelial sodium channels. J Biol Chem 278:36202–36213

Chen SY, Bhargava A, Mastroberardino L, Meijer OC, Wang J, Buse P, Firestone GL, Verrey F, Pearce D (1999) Epithelial sodium channel regulated by aldosterone-induced protein sgk. Proc Natl Acad Sci USA 96:2514–2519

Chyb S, Raghu P, Hardie RC (1999) Polyunsaturated fatty acids activate the *Drosophila* light-sensitive channels TRP and TRPL. Nature 397:255–259

Denson DD, Wang X, Worrell RT, Eaton DC (2000) Effects of fatty acids on BK channels in GH(3) cells. Am J Physiol Cell Physiol 279:C1211–C1219

Eaton DC, Malik B, Saxena NC, Al-Khalili OK, Yue G (2001) Mechanisms of aldosterone's action on epithelial Na^+ transport. J Membr Biol 184:313–319

Els WJ, Helman SI (1997) Dual role of prostaglandins (PGE2) in regulation of channel density and open probability of epithelial Na^+ channels in frog skin (*R. pipiens*). J Membr Biol 155:75–87

Farman N, Pradelles P, Bonvalet JP (1986) Determination of prostaglandin E2 synthesis along rabbit nephron by enzyme immunoassay. Am J Physiol 251:F238–F244

Friedrich B, Feng Y, Cohen P, Risler T, Vandewalle A, Broer S, Wang J, Pearce D, Lang F (2003) The serine/threonine kinases SGK2 and SGK3 are potent stimulators of the epithelial Na^+ channel alpha, beta, gamma-ENaC. Pflugers Arch 445:693–696

Goodman DB, Wong M, Rasmussen H (1975) Aldosterone-induced membrane phospholipid fatty acid metabolism in the toad urinary bladder. Biochemistry 14:2803–2809

Gorelik J, Gu Y, Spohr HA, Shevchuk AI, Lab MJ, Harding SE, Edwards CR, Whitaker M, Moss GW, Benton DC, Sanchez D, Darszon A, Vodyanoy I, Klenerman D, Korchev YE (2002) Ion channels in small cells and subcellular structures can be studied with a smart patch-clamp system. Biophys J 83:3296–3303

Guan Y, Zhang Y, Breyer RM, Fowler B, Davis L, Hebert RL, Breyer MD (1998) Prostaglandin E2 inhibits renal collecting duct Na^+ absorption by activating the EP1 receptor. J Clin Invest 102:194–201

Hebert RL, Breyer RM, Jacobson HR, Breyer MD (1995) Functional and molecular aspects of prostaglandin E receptors in the cortical collecting duct. Can J Physiol Pharmacol 73:172–179

Hebert RL, Carmosino M, Saito O, Yang G, Jackson CA, Qi Z, Breyer RM, Natarajan C, Hata AN, Zhang Y, Guan Y, Breyer MD (2005) Characterization of a rabbit kidney prostaglandin F(2{alpha}) receptor exhibiting G(i)-restricted signaling that inhibits water absorption in the collecting duct. J Biol Chem 280:35028–35037

Hill WG, An B, Johnson JP (2002) Endogenously expressed epithelial sodium channel is present in lipid rafts in A6 cells. J Biol Chem 277:33541–33544

Hornych A, Bedrossian J, Bariety J, Menard J, Corvol P, Safar M, Fontaliran F, Milliez P (1975) Prostaglandins and hypertension in chronic renal diseases. Clin Nephrol 4:144–151

Kim D, Clapham DE (1989) Potassium channels in cardiac cells activated by arachidonic acid and phospholipids. Science 244:1174–1176

Kokko KE, Matsumoto PS, Ling BN, Eaton DC (1994) Effects of prostaglandin E2 on amiloride-blockable Na^+ channels in a distal nephron cell line (A6). Am J Physiol 267:C1414–C1425

Lazzeri M, Barbanti G, Beneforti P, Maggi CA, Taddei I, Andrea U, Cantini C, Castellani S, Turini D (1995) Vesical–renal reflex: diuresis and natriuresis activated by intravesical capsaicin. Scand J Urol Nephrol 29:39–43

Ma HP, Saxena S, Warnock DG (2002) Anionic phospholipids regulate native and expressed epithelial sodium channel (ENaC). J Biol Chem 277:7641–7644

Makita K, Takahashi K, Karara A, Jacobson HR, Falck JR, Capdevila JH (1994) Experimental and/or genetically controlled alterations of the renal microsomal cytochrome P450 epoxygenase induce hypertension in rats fed a high salt diet. J Clin Invest 94:2414–2420

Matsumoto PS, Mo L, Wills NK (1997) Osmotic regulation of Na^+ transport across A6 epithelium: interactions with prostaglandin E2 and cyclic AMP. J Membr Biol 160:27–38

Michaelis UR, Fisslthaler B, Barbosa-Sicard E, Falck JR, Fleming I, Busse R (2005) Cytochrome P450 epoxygenases 2C8 and 2C9 are implicated in hypoxia-induced endothelial cell migration and angiogenesis. J Cell Sci 118:5489–5498

Narumiya S (1994) Prostanoid receptors. Structure, function, and distribution. Ann N Y Acad Sci 744:126–138

Nielsen R (1990) Prostaglandin E2 enhances the sodium conductance of exocrine glands in isolated frog skin (*Rana esculenta*). J Membr Biol 113:31–38

Nusing RM, Seyberth HW (2004) The role of cyclooxygenases and prostanoid receptorsin furosemide-like salt losing tubulopathy: the hyperprostaglandin E syndrome. Acta Physiol Scand 181:523–528

Oliver D, Lien CC, Soom M, Baukrowitz T, Jonas P, Fakler B (2004) Functional conversion between A-type and delayed rectifier K^+ channels by membrane lipids. Science 304:265–270

Ordway RW, Walsh JV Jr, Singer JJ (1989) Arachidonic acid and other fatty acids directly activate potassium channels in smooth muscle cells. Science 244:1176–1179

Smith WL (1992) Prostanoid biosynthesis and mechanisms of action. Am J Physiol 263:F181–F191

Smith WL, Marnett LJ, DeWitt DL (1991) Prostaglandin and thromboxane biosynthesis. Pharmacol Ther 49:153–179

Staruschenko A, Nichols A, Medina JL, Camacho P, Zheleznova NN, Stockand JD (2004a) Rho small GTPases activate the epithelial Na(+) channel. J Biol Chem 279:49989–49994

Staruschenko A, Patel P, Tong Q, Medina JL, Stockand JD (2004b) Ras activates the epithelial Na(+) channel through phosphoinositide 3-OH kinase signaling. J Biol Chem 279:37771–37778

Sugimoto Y (2006) Expression and function of prostanoid receptors. Seikagaku 78:1039–1049

Sugimoto Y, Narumiya S (2007) Prostaglandin E receptors. J Biol Chem 282:11613–11617

Sun P, Lin DH, Wang T, Babilonia E, Wang Z, Jin Y, Kemp R, Nasjletti A, Wang WH (2006) Low Na intake suppresses expression of CYP2C23 and arachidonic acid–induced inhibition of ENaC. Am J Physiol Renal Physiol 291:F1192–F1200

Vallet V, Chraibi A, Gaeggeler HP, Horisberger JD, Rossier BC (1997) An epithelial serine protease activates the amiloride-sensitive sodium channel. Nature 389:607–610

Wegmann M, Nusing RM (2003) Prostaglandin E2 stimulates sodium reabsorption in MDCK C7 cells, a renal collecting duct principal cell model. Prostaglandins Leukot Essent Fatty Acids 69: 315–322

Wei Y, Lin DH, Kemp R, Yaddanapudi GS, Nasjletti A, Falck JR, Wang WH (2004) Arachidonic acid inhibits epithelial Na channel via cytochrome P450 (CYP) epoxygenase-dependent metabolic pathways. J Gen Physiol 124:719–727

Wei Y, Sun P, Wang Z, Yang B, Carroll MA, Wang WH (2006) Adenosine inhibits ENaC via cytochrome P-450 epoxygenase-dependent metabolites of arachidonic acid. Am J Physiol Renal Physiol 290:F1163–F1168

Worrell RT, Bao HF, Denson DD, Eaton DC (2001) Contrasting effects of cPLA2 on epithelial Na^+ transport. Am J Physiol Cell Physiol 281:C147–C156

Wu KK, Liou JY (2005) Cellular and molecular biology of prostacyclin synthase. Biochem Biophys Res Commun 338: 45–52

Yorio T, Bentley PJ (1978) Phospholipase A and the mechanism of action of aldosterone. Nature 271:79–81

Yue G, Malik B, Yue G, Eaton DC (2002) Phosphatidylinositol 4,5-bisphosphate (PIP2) stimulates epithelial sodium channel activity in A6 cells. J Biol Chem 277:11965-11969