Effect of Arachidonic Acid on Activity of the Apical K^+ Channel in the Thick Ascending Limb of the Rat Kidney

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ABSTRACT We have used patch-clamp techniques to study the effects of arachidonic acid (AA) on the activity of the 70-pS K^+ channel, the predominant type of the two apical K^+ channels operating under physiological conditions in the thick ascending limb (TAL) of the rat kidney. Addition of 5–10 μM AA blocked the activity of the 70-pS K^+ channel in both cell-attached and inside-out patches. The inhibitory effect of AA was specific, because application of 10 μM linoleic acid, oleic acid, or palmitic acid failed to mimic the effect of AA. The effect of AA could not be blocked by pretreatment of the TAL tubules with either 5 μM indomethacin (inhibitor of cyclooxygenase) or 4 μM cinnamyl-3,4-dihydroxy-α-cyanocinnamate (CDC) (inhibitor of lipooxygenase). In contrast, addition of 5 μM 17-octadecynoic acid (17-ODYA), an inhibitor of P450 monooxygenases, abolished the effect of AA on the channel activity, indicating that the effect was mediated by cytochrome P450 metabolites of AA. Addition of 10 nM 20-hydroxyeicosatetraenoic acid (20-HETE), the main metabolite of the cytochrome P450 metabolic pathway in the medullary TAL, mimicked the inhibitory effect of 10 μM AA. However, addition of 100 nM 19-HETE or 17-HETE had no significant effects and 100 nM 20-carboxy AA (20-COOH) reduced the channel activity by only 20%, indicating that the inhibitory effect of 20-HETE was specific and responsible for the action of AA. Inhibition of the P450 metabolic pathway by either 5 μM 17-ODYA or 12, 12-dibromododec-11-enoic acid (DBDD) dramatically increased the channel activity by 280% in cell-attached patches. The stimulatory effect of 17-ODYA or DBDD was not observed in inside-out patches. The results strongly indicate that 20-HETE is a specific inhibitor for the 70-pS K^+ channel and may play an important role in the regulation of the K^+ channel activity in the TAL.

INTRODUCTION

Apical K^+ channels play an important role in the reabsorption of NaCl in the thick ascending limb (TAL) by providing a route for recycling K^+ across the apical membrane, a process that is essential for sustaining the normal function of the $Na^+/2Cl^-/K^+$ cotransporter (Greger, 1985; Stanton and Giebisch, 1992; Molony, Reeves, and Andreoli, 1989; Hebert and Andreoli, 1984). The importance of the

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K^+ recycling across the apical membrane of the TAL has been shown in experiments in which inhibition of the apical K^+ conductance with Ba^{2+} reduced the short circuit current by 50% (Greger, and Schlatter, 1981) and removal of luminal K^+ in the presence of Ba^{2+} completely abolished the short circuit current, indicating that the Na^+ reabsorption was blocked. Similar results were also observed in the isolated perfused mouse TAL in which application of Ba^{2+} and the removal of luminal K^+ abolished net Cl^- reabsorption (Hebert and Andreoli, 1984). In addition, glyburide, which blocks the ATP-sensitive K^+ channel in several tissues (Edwards and Weston, 1993), has a natriuretic effect (Clark, Humphrey, Smith, and Ludens, 1993).

Three types of K^+ channels, a Ca^{2+}-activated "maxi" K^+ channel, an ATP-sensitive 30-pS and an ATP-sensitive 70-pS K^+ channel, have been identified in the apical membrane of the mammalian TAL (Bleich, Schlatter, and Greger, 1990; Wang, White, Geibel, and Giebisch, 1990; Taniguchi and Guggino, 1989; Wang, 1994). However, there is a consensus that the Ca^{2+}-activated "maxi" K^+ channel is not responsible for the K^+ recycling, since the channel open probability is $<1\%$ under control conditions and the likelihood of finding a functional "maxi" K^+ channel is very low ($<1\%$) in the native mammalian tissue. The "maxi" K^+ channel may be important for the regulation of cell volume, because activity of the "maxi" K^+ channel is increased in response to cell swelling (Taniguchi and Guggino, 1989). Thus, it is most likely that the 70-pS K^+ channel and the 30-pS^+ channel are responsible for K^+ recycling across the apical membrane of TAL (Wang, 1994).

Arachidonic acid (AA) and its metabolites play an important role in the regulation of electrolyte and fluid transport in the kidney (Hirt, Capdevila, Falck, Breyer, and Jacobson, 1989; Ling, Webster, and Eaton, 1992; Escalante, Erlij, Falck, and McGiff, 1991; Friedlander, Grimellec, Sraer, and Amiel, 1990; Zou, Imig, Ortiz de Montellano, Sui, Falck, and Roman, 1994; Pedrosa-Ribeiro, Dubay, Falck, and Mandel, 1994). It was demonstrated that AA decreased Rb^+ uptake in the isolated TAL and the cytochrome P450 metabolite, 20-hydroxyeicosatetraenoic acid (20-HETE), mimicked the effects of AA, suggesting that the effects of AA on Rb^+ uptake were mediated by cytochrome P450 metabolites of AA. The inhibitory effects of P450 metabolites on Rb^+ uptake were also observed after inhibition of Na^+-K^+-ATPase by ouabain, suggesting that the effect of the P450 metabolites on Rb^+ uptake was a result of inhibition of luminal entry. This view was further supported by experiments in which addition of furosemide to block the cotransporter largely abolished the effects of P450 metabolites on the Rb^+ uptake (Escalante et al., 1991). However, the mechanism by which P450 metabolites inhibit the cotransporter is not completely understood, because a decrease in the activity of the cotransporter can be achieved by either its direct inhibition or by blockade of the K^+ recycling. The present experiments were designed to explore further the effect of AA and its P450 metabolites on the activity of the 70-pS K^+ channels in the apical membrane of the TAL.

METHODS

Preparation of TAL

Pathogen-free Sprague-Dawley rats (80–120 g) of either sex (Taconic Inc., Germantown, NY) were maintained on control rat chow. Animals were anesthetized with diethyl ether and then decapi-

tated. After being killed, both kidneys were immediately removed and 1 mm thin coronal sections cut with a razor blade. The cortical or medullary TAL tubules were dissected in HEPES-buffered Ringer solution containing (in millimolar): 135 NaCl; 5 KCl; 1.5 $MgCl_2$; 1.8 $CaCl_2$; 5 glucose; and 10 HEPES (pH 7.4 with NaOH) at 22°C and the tubules were placed on a cover glass coated with "Cell-Tak" (Collaborative Research Inc., Bedford, MA). The TALs on the cover glass were placed in a chamber (1,200 μl) mounted on an inverted microscope (Nikon) and superfused with HEPES-buffered Ringer solution. The tubules were cut open with a sharpened micropipette to expose the apical membrane. The temperature of the chamber were maintained at 37 ± 1°C by circulating warm water surrounding the chamber.

Patch-Clamp Technique

Patch-clamp experiments were described previously (Wang, Geibel, and Giebisch, 1993; Wang and Giebisch, 1991; Wang, Schwab, and Giebisch, 1990). Briefly, patch-clamp pipettes were pulled from glass capillaries (Degan, Minneapolis, MN) and had resistances of 4–6 MΩ when filled with 140 mM KCl. Recordings were made with an Axon 200A patch-clamp amplifier and single-channel currents were low-pass filtered at 1 KHz using an eight-pole Bessel filter (No. 902LPF, Frequency Devices, Inc., Haverhill, MA). The recordings were digitized at a sampling rate of 44 KHz using a modified Sony PCM-501ES pulse code modulator and stored on videotape (Sony SL-2700). For analysis, the data were transferred to an IBM-compatible 486 computer (Gateway 2000) at a rate of 5 kHz and analyzed using the pClamp software system 6.0 (Axon Instruments, Inc., Burlingame, CA).

Opening and closing transitions were detected using 50% of the single-channel amplitude as the threshold. In the present experiments, we did not try to determine the channel number (N) and channel open probability (P_o), because it is very difficult to calculate the true channel number in a patch with multiple channels. To bypass this difficulty, we employed NP_o as a measurement for the channel activity. The NP_o is a product between the channel number in the given patch and the P_o. An alteration of NP_o could be achieved by changes in either N or P_o. The NP_o was calculated from data samples of 60-s duration under control conditions and after reaching steady state as follows:

$$NP_o = \Sigma (t_1 + t_2 + \dots t_n) \quad (1)$$

where $t_{x(x=1, 2,\dots n)}$ is the ratio between time observing open-channel current and the total time of measurement at each of the current levels. The steady state was determined when the NP_o was maintained constantly for 60 s after the onset of the action of a particular agent. However, if no effect of a given agent was observed in 15 min, the NP_o of the last minute was calculated as the steady state. To calculate the total apparent channel number it was essential to determine the channel close line from each experiment. If the channel closed level was not detected, 1 mM Ba^{2+} was added at the end of experiments to determine the base line.

Experimental Solutions and Chemicals

Arachidonic acid, oleic acid, and indomethacin were purchased from Sigma Chemical Co. (St. Louis, MO) and linoleic acid and 16-hydroxyhexadecanoic acid (palmitic acid) were purchased from Cayman Chemical Co. (Ann Arbor, MI). DBDD, 20-, 19-, 17-hydroxyeicosatetraenoic acid (HETE) and 20-carboxyl arachidonic acid were obtained from Dr. Falck (University of Texas Health Science Center, Dallas, TX). 17-ODYA and CDC were obtained from Biomolecular Research Laboratories (Plymouth Meeting, PA). The chemicals were dissolved in ethanol (50%) and added directly to the bath to achieve the designed concentration. The pipette solution was composed of 140 mM KCl, 1.8 mM $MgCl_2$, 1 mM EGTA, and 10 mM HEPES (pH 7.4) and the bath solution for inside-out patches contained 140 mM NaCl, 5 mM NaCl, 1.8 mM $MgCl_2$, 100 μM MgATP, 1 mM EGTA, and 10 mM HEPES (pH 7.4).

Statistics

Data are presented as mean ± SEM and paired *t* test was used to determine the statistical significance. $*P < 0.05$.

RESULTS

We have confirmed previous findings that two types of K^+ channels, a 30-pS and a 70-pS, coexist in the apical membrane in both the medullary and the cortical thick ascending limb (TAL) under physiological conditions (Wang, 1994). In 188 experiments (forming a GΩ seal on the apical membrane), we have observed the 70-pS K^+ channel in 75 patches and the 30-pS K^+ channel in 54 patches. However, the average NP_o of the 70-pS K^+ channel was 1.5 ± 0.2 whereas the average NP_o of the 30-pS K^+ channel was 0.8 ± 0.1. It is apparent that the 70-pS K^+ channel is mainly responsible for the apical K^+ conductance. Accordingly, in this study we have focused on exploring the regulatory mechanism of the 70-pS K^+ channel in the apical membrane of the TAL. Because the biophysical properties of the apical K^+ channels observed in the cortical TAL were identical to that in the medullary TAL, we have pooled the data.

AA has been shown to inhibit the renal ATP-sensitive K^+ channel in the cortical collecting duct (Wang, Cassola, and Giebisch, 1992). In the present experiments, we have examined whether AA also blocked the activity of the 70 pS K^+ channel. Fig. 1 shows a representative recording demonstrating the effect of 5 μM AA on the activity of the 70-pS K^+ channel in an inside-out patch. The activity of the 70-pS K^+ channel was completely and reversibly blocked by 10 μM AA (Table I)and the effects of AA were observed in both cell-attached and inside-out patches. Fig. 2 further shows the dose response curve of the effects of AA on the channel activity, showing that the concentration of AA required to block the channel activity by 50% was ~3 μM.

It is well known that the properties of the lipid membrane could affect the function of membrane proteins, such as ion channels, by alteration of either membrane fluidity or membrane deformation energy (Kimelberg and Papahadjopoulos, 1974; Harris, 1985; Lundbæk and Anderson, 1994). To determine whether the effect of AA resulted from a change in the membrane properties, we examined the effects of other fatty acids on the activity of the 70-pS K^+ channel in inside-out patches and the results are summarized in Table II. Addition of either 10 μM linoleic acid or

TABLE I

Effect of AA, 20-HETE and 17-ODYA/DBDD on the Channel Activity

	AA	AA	20-HETE	20-HETE	17-ODYA/DBDD
Concentration	*5 μM*	*10 μM*	*5 nM*	*10 nM*	*5 μM*
Percent of the control	7 ± 2%*	0%*	6 ± 1%*	1 ± 1*	280 ± 30%*
N	22	18	6	7	8

*Data that are statistically significant in comparison to the control value. The experiments with 17-ODYA/DBDD were carried out in cell-attached patches.

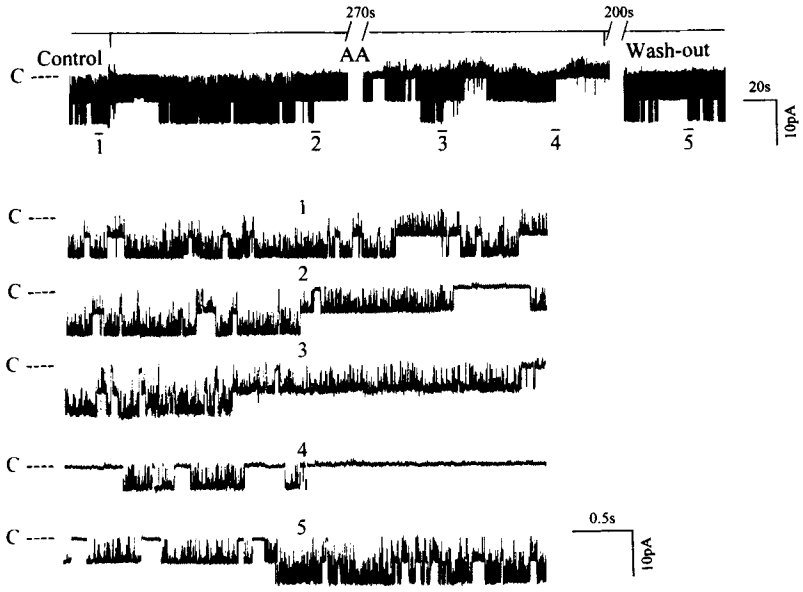

FIGURE 1. A single-channel recording made in an inside-out patch showing the effect of 5 μM AA on the activity of the 70-pS K^+ channel in the TAL. The bath solution contained 140 mM NaCl, 5 mM KCl, 1.8 mM $MgCl_2$, 1 mM EGTA, 100 μM MgATP and 10 mM HEPES (pH 7.4) and the pipette solution was composed of 140 mM KCl, 1.8 mM $MgCl_2$, 1 mM EGTA and 10 mM HEPES (pH 7.4). The upper panel of the figure shows the channel recording at slow time course and five parts of the traces indicated by a short bar and number are extended at fast time resolution in the lower panel. The channel closed states are indicated by *C* and the cell membrane potential was 0 mV.

oleic acid (*cis* unsaturated fatty acids) or 10 μM palmitic acid (a saturated fatty acid) failed to mimic the inhibitory effect of AA, suggesting that the effect of AA on the channel activity was a specific effect.

Having demonstrated that AA inhibited the activity of the 70-pS K^+ channel in the TAL, we next examined whether the action of AA was a direct effect or an effect that was mediated by its metabolites. Thus, the effect of AA on the channel activity was further examined after inhibition of AA metabolism. AA can be metabolized in the kidney by three sets of enzymes, lipoxygenase, cyclooxygenase, and cytochrome P450 oxygenase (McGiff, 1991). We first examined the effect of AA on channel activity in the presence of 4 μM CDC ($n = 4$), an inhibitor of lipoxygenase (Fig. 3). Addition of CDC had no significant effect on the activity of the 70-pS K^+ channel. Furthermore, inhibition of lipoxygenase failed to block the effect of AA on the channel activity, because 5 μM AA still decreased the channel activity to the same extent in the presence of CDC (9 ± 2% of the control value, $n = 4$) as that observed without CDC (7 ± 2% of the control value, $n = 22$), indicating that the effect of AA was not mediated by lipoxygenase-dependent metabolites. Second, the inhibitory effect of AA on the activity of the 70-pS K^+ channel was investigated in the presence of 5 μM indomethacin, an inhibitor of cyclooxygenase. As in the case of the lipoxygenase inhibitor, inhibition of cyclooxygenase failed to block the in-

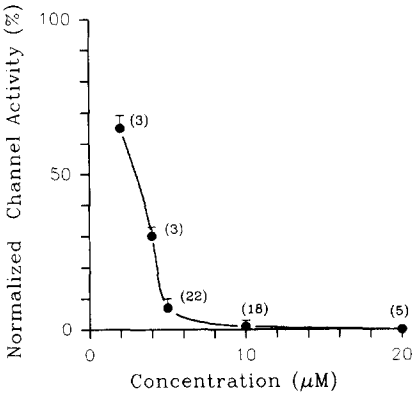

FIGURE 2. A dose-response curve of the effect of AA on channel activity in inside-out patches.

hibitory effect of AA. In the presence of indomethacin, 5 μM AA reduced the channel activity to 7 ± 2% of the control value (n = 5), which is not different from the value obtained without indomethacin. Finally, we studied the effect of AA on the channel activity in the presence of 5 μM 17-ODYA, a potent inhibitor of P450 monooxygenase. Fig. 4 is a typical recording made in an inside-out patch showing the effect of AA after inhibition of P450 monooxygenase (n = 7). It is apparent that 17-ODYA had no significant effect on the activity of the 70-pS K^+ channel in inside-out patches. However, the effect of AA on the channel activity was completely abolished in the presence of 17-ODYA (Fig. 4), indicating that the effect of AA on the activity of the 70-pS K^+ channel was mediated by cytochrome P450 metabolites. The observations that the effect of AA on channel activity can be fully blocked by 17-ODYA in inside-out patches suggest that P-450 metabolic enzymes were present in the patch membrane. It was demonstrated using electron microscopy that the patch is not a bare bilayer and may contain some organelles and cytoskeleton (Ruknudin, Song, and Sachs, 1991). Because the P-450 metabolic enzymes are bound to the membrane of microsomes, it is conceivable that the microsomes may be attached to the apical membrane of the TAL cells.

It was demonstrated that 20-HETE and 20-COOH were the main products of the cytochrome-P450-dependent metabolic pathway in the medullary TALs in the rabbit kidney (Escalante et al., 1991). We next examined the effect of 20-HETE on the activity of the 70-pS K^+ channel. Fig. 5 is a representative recording made in an inside-out patch showing the effect of 20-HETE on channel activity. It is apparent that addition of 5 nM 20-HETE mimicked the effect of AA, decreasing channel ac-

TABLE II

Effect of Fatty Acids on the Eicosanoids on the Activity of the 70-pS K^+ Channel

	OA	LA	PA	19-HETE	20-COOH	17-HETE
Concentration	*10 μM*	*10 μM*	*10 μM*	*100 nM*	*100 nM*	*100 nM*
Percent of the control	93 ± 5	92 ± 6	95 ± 4	95 ± 2	80 ± 3*	95 ± 2
N	5	5	3	3	4	3

*Data that are statistically significant in comparison to the control value.

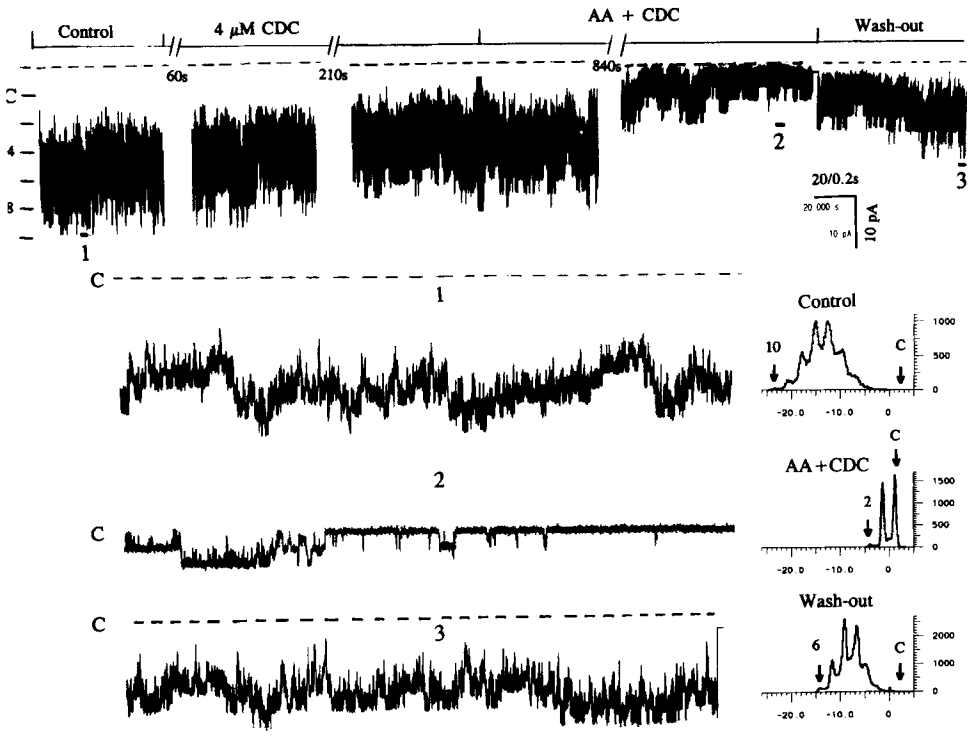

FIGURE 3. A recording showing the effect of 5 μM AA on the activity of the 70-pS K^+ channel in an inside-out patch in the presence of 4 μM CDC. The cell membrane potential was 0 mV and the bath and pipette solutions were the same as described in Fig. 1. The top shows the channel recording at slow time course and three parts of the traces indicated by a short bar and number are extended at fast time resolution in the bottom. The right side of the figure shows the all-points amplitude histogram. The number and arrow indicate the maximum channel number and channel closed level, respectively. The channel closed-states are indicated by *C* and the cell membrane potential was 0 mV.

tivity to 6 ± 1% of the control value (n = 6). The inhibitory effect of 20-HETE was reversible and wash-out restored the channel activity. Inspection of Figs. 1 and 5 shows that the onset of 20-HETE action was significantly faster (75 ± 10 s) than that of AA (360 ± 50 s). The view that 20-HETE may mediate the effect of AA was further supported by observing the inhibitory effect of 20-HETE in the presence of inhibitors of P450 monooxygenase (Fig. 6). It is apparent that the effect of 20-HETE on channel activity is not different with DBDD or 17-ODYA or without the inhibitors. The effect of 20-HETE on the channel activity was also observed in cell-attached patches with a similar time course (data not shown). Fig. 7 is a dose-response curve for the effect of 20-HETE on channel activity; the estimated K_d for 20-HETE is 2.5 nM. In contrast, application of 5 nM 20-COOH had no effect on the activity of the 70-pS K^+ channel (data not shown), and a higher concentration (100 nM) decreased the channel activity by only 20 ± 3% (Table II). Having established that 20-HETE was the main mediator for AA-induced inhibition of the 70-pS K^+ in the TAL, we examined the effects of 19-HETE and 17-HETE to determine the spec-

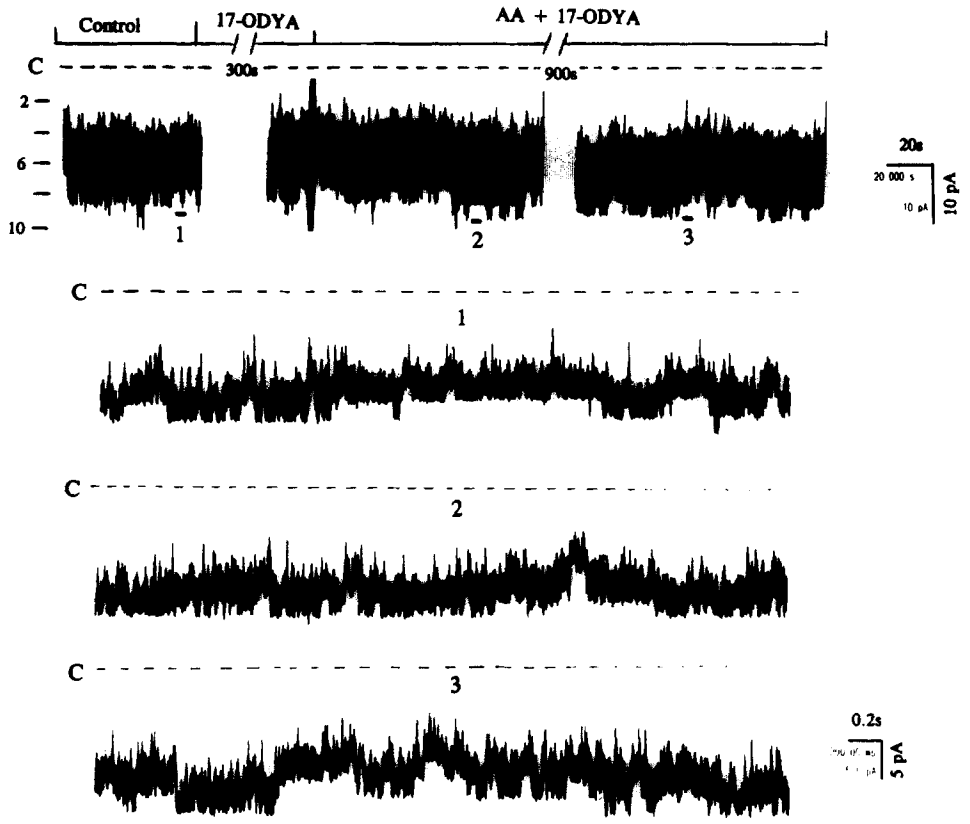

FIGURE 4. A recording showing the effect of 5 μM AA on the activity of the 70-pS K^+ channel in an inside-out patch in the presence of 5 μM 17-ODYA. The cell membrane potential was 0 mV and the bath and pipette solutions were the same as described in Fig. 1. The top shows the channel recording at slow time course and three parts of the traces indicated by a short bar and number are extended at fast time resolution in the bottom. The channel closed states are indicated by *C* and the cell membrane potential was 0 mV.

ificity of the effect of 20-HETE. Table II summarizes the results from three experiments. In contrast to 20-HETE, neither 100 nM 19-HETE nor 17-HETE had any significant effect on the activity of the 70-pS K^+ channel, indicating that the effect of 20-HETE is highly specific and that the agent is a main mediator for the AA action.

Although we have demonstrated that the cytochrome P450 metabolite of AA, 20-HETE, inhibits the 70-pS K^+ channel in the TAL, its role in the regulation of the apical K^+ channels under physiological conditions is not known. Fig. 8 is a representative recording made in a cell-attached patch out of five experiments to show the effects of inhibiting the P450 metabolic pathway. Before addition of 17-ODYA, only one channel current level was observed; addition of 5 μM 17-ODYA dramatically increased the channel activity. Several inhibitors of P450 metabolism are known to have an effect on channel activity through a direct action not related to

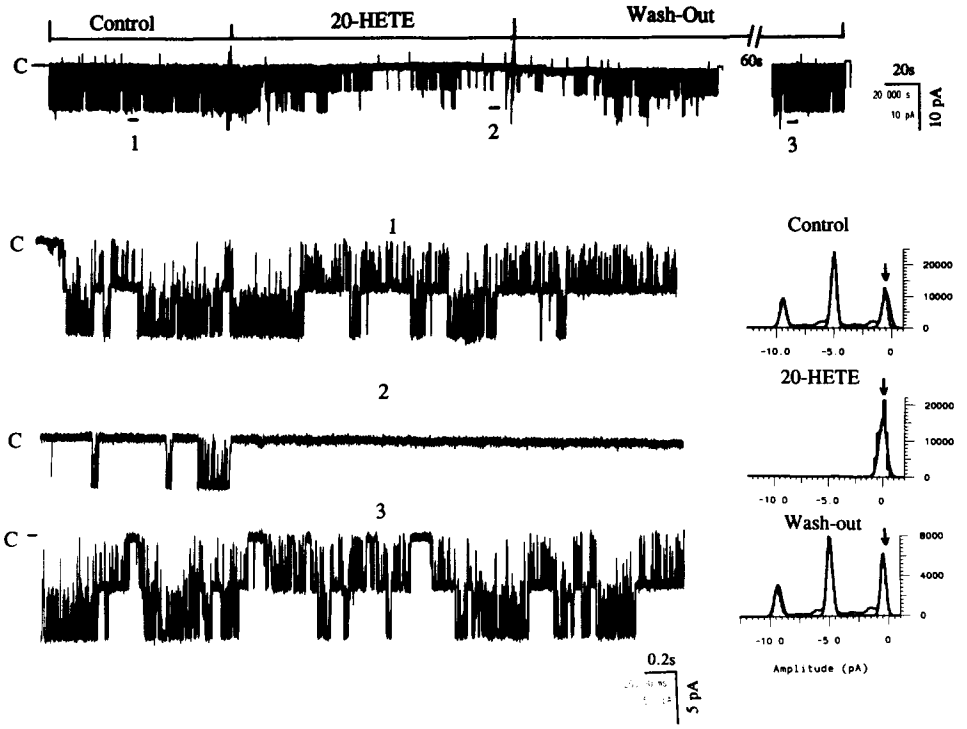

FIGURE 5. A recording showing the effect of 5 nM 20-HETE on the activity of the 70-pS K^+ channel in an inside-out patch. The cell membrane potential was 0 mV and the bath and pipette solutions were the same as described in Fig. 1. The top of the figure shows the channel recording at slow time course and three parts of the traces indicated by a short bar and number are extended at fast time resolution in the bottom. The right side of the figure shows the all-points amplitude histogram and arrow indicates the channel closed level. The channel closed-states are indicated by *C* and the cell membrane potential was 0 mV.

the inhibition of the enzymes (McManus, Serhan, Jackson, and Strange, 1994; Sakuta and Yoneda, 1994). The fact that addition of 17-ODYA to the bath had no effect on the channel activity in inside-out patches (Fig. 4), indicates that its effect in the cell-attached configurations is not a direct one. However, it is conceivable that the inhibitor might exert its action in cell-attached patches by a mechanism other than inhibition of P450 metabolism in cell-attached patches. To exclude this possibility, we examined the effect on the channel activity of another structurally unrelated inhibitor of P450 monooxygenase, DBDD in three separate experiments. Fig. 9 shows that addition of DBDD also stimulated the activity of the 70-pS K^+ channel in cell attached patches. In eight experiments, inhibition of the P450 metabolic pathway of AA with either 17-ODYA or DBDD increased the channel activity by 280 ± 30% (Table I), indicating that 20-HETE is present in the TAL cells and involved in the regulation of the activity of the apical K^+ conductance.

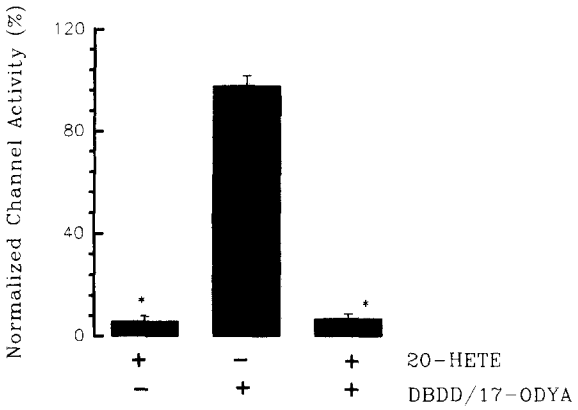

FIGURE 6. A diagram showing the effect of 5 nM 20-HETE in the presence of 17-ODYA/DBDD ($N = 3$) or in the absence of the inhibitor of P450 monooxygenase.

DISCUSSION

The TAL absorbs 20–25% of the filtered Na^+ and is essential for the urinary concentration mechanism (Greger, 1985; Stanton and Giebisch, 1992). The active NaCl reabsorption in TAL occurs by two separate steps. First, Na^+ and Cl^- enter the cell across the apical membrane through a Na/2Cl/K cotransporter energized by a favorable Na^+ and Cl^- gradient. Second, Na^+ is pumped out of the cell across the basolateral membrane via Na-K-ATPase; Cl^- diffuses from the cell to the peritubular side through a Cl^- channel or a KCl symporter (Greger, 1985; Hebert and Andreoli, 1984; Molony, Reeves, and Andreoli, 1989). Apical K^+ channels are responsible for recycling K^+ across the apical membrane in the TAL, a process that is essential for maintaining the normal function of the Na/2Cl/K cotransporter and, accordingly, for the reabsorption of Na^+. First, recycling K^+ across the luminal membrane is partially responsible for the lumen positive transepithelial potential, which is the driving force for the paracellular reabsorption of Na^+. Second, K^+ leaving the cell across the apical membrane hyperpolarizes the apical as well as basolateral membrane and thus provides an important driving force for Cl^- to diffuse to the peritubular side. Finally, recycling K^+ is important for providing an adequate

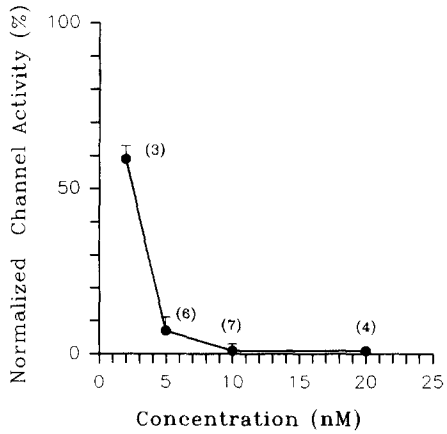

FIGURE 7. A dose response curve of the effect of 20-HETE on channel activity in inside-out patches.

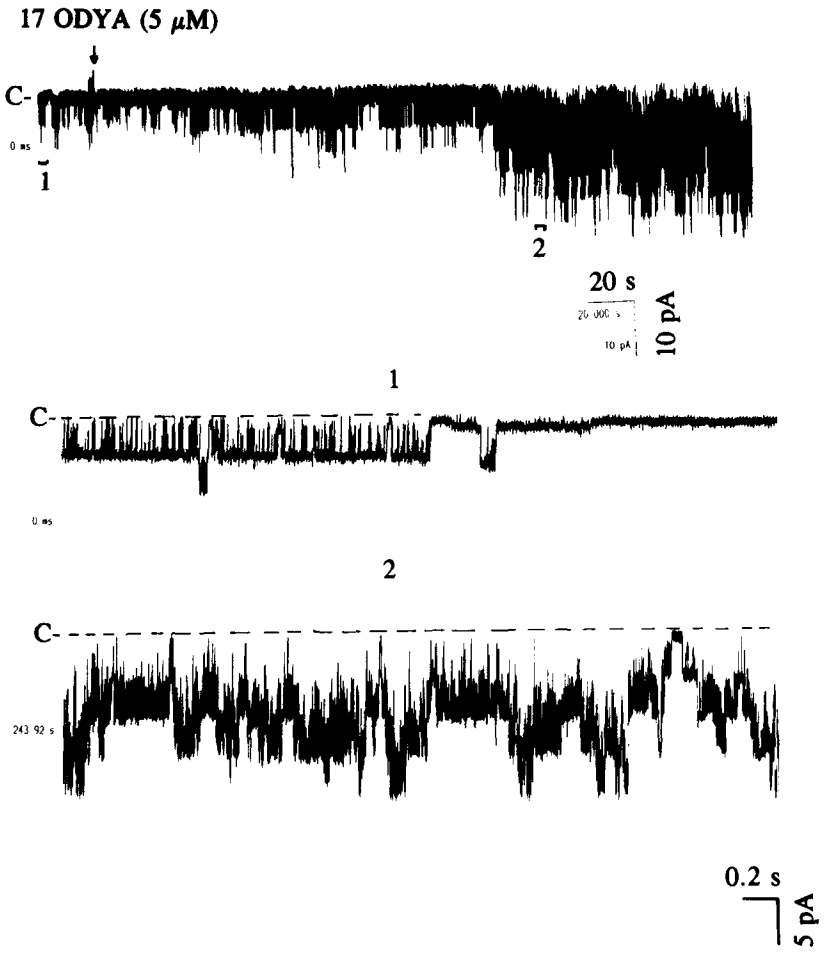

FIGURE 8. A channel recording showing the effect of 5 μM 17-ODYA on the activity of the 70-pS K^+ channel in a cell-attached patch. The upper panel of the figure shows the channel recording at slow time course and two parts of the traces indicated by a short bar and number are extended at fast time resolution in the lower panel. The channel closed-states are indicated by *C* and the cell membrane potential was 0 mV.

supply of K^+ to the cotransporter, because the luminal Na^+ and Cl^- concentrations are at least one order of magnitude higher than the luminal K^+ concentration (Greger, 1985; Stanton and Giebisch, 1992). Thus, the regulation of the activity of apical K^+ channels is an important step in the overall regulation of NaCl reabsorption in the TAL.

In the present study we have demonstrated that AA through its P450 metabolites is involved in the modulation of the activity of the 70-pS K^+ channel in the TAL. Our unpublished observations demonstrated that AA also blocked the activity of the 30-pS K^+ channel. Three possible mechanisms might explain the effects of AA

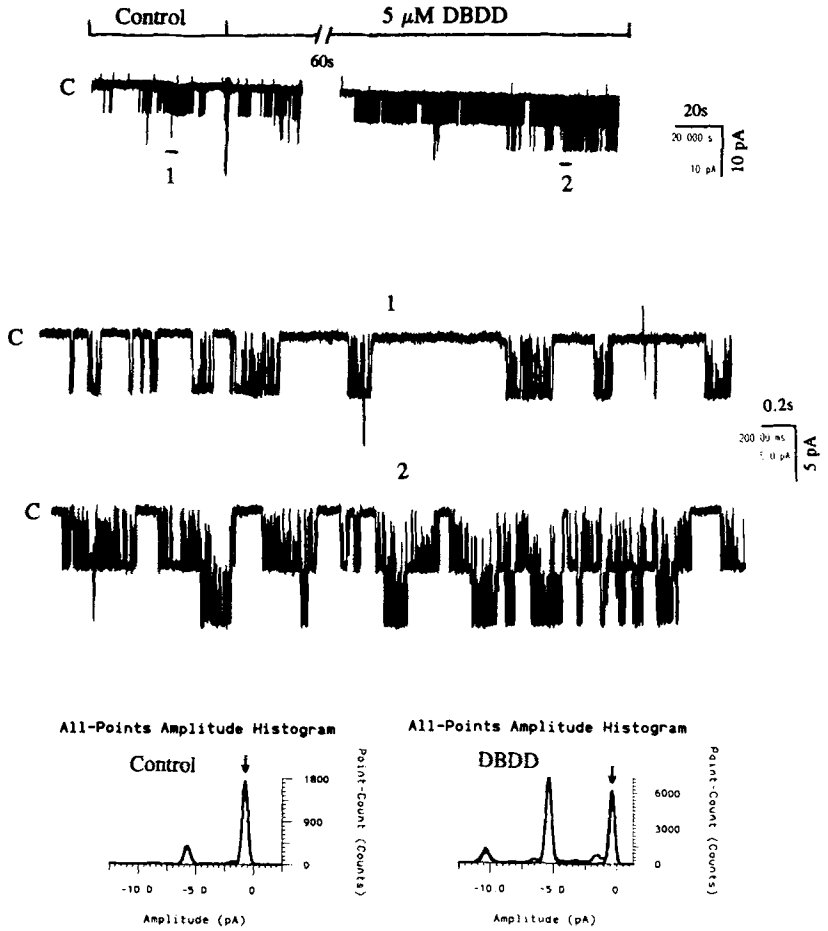

FIGURE 9. A channel recording showing the effect of 5 μM DBDD on the activity of the 70-pS K^+ channel in a cell-attached patch. The upper panel of the figure shows the channel recording at slow time course and two parts of the traces indicated by a short bar and number are extended at fast time resolution in the middle. The channel closed-states are indicated by *C* and the cell membrane potential was 0 mV. The lower panel of the figure shows the all-points amplitude histogram and arrow indicates the channel closed level.

and its metabolites. First, AA could alter the membrane fluidity and, consequently, the function of the K^+ channel (Grimellec, 1992). Second, AA may change the function of the K^+ channel by altering the membrane deformation energy (Lundbæk and Andersen, 1994). Finally, the AA metabolites may either directly affect the channel activity or indirectly modulate the K^+ channel through mediation of the membrane delimited signal transduction pathways (Ordway, Walsh, and Singer, 1989; Ordway, Singer, and Walsh, Jr., 1991). Changes in the membrane fluidity modify the function of several membrane proteins such as Na^+-K^+-ATPase in the kidney (Kimelberg and Papahadjopoulos, 1974; Harris, 1985). However, it is un-

likely that the effects of P450 metabolites of AA on the activity of the 70-pS K^+ channel were a result of changes in the membrane fluidity, because the effect of AA was not mimicked by *cis* unsaturated fatty acids, such as oleic acid (OA) and linoleic acid. It has been demonstrated that 10 μM OA-induced perturbation of cell membrane lipid order is very similar to that induced by 5 μM AA in T lymphocytes (Richierl, Mescher, and Kleinfeld, 1990). However, addition of 10 μM OA has no effect on channel activity whereas 5 μM AA almost completely blocked the channel activity. Furthermore, if the effects of AA were mediated by alterations of the membrane fluidity, saturated and unsaturated fatty acids should have opposite effects (Grimellec, 1992). However, we found that neither palmitic acid (saturated fatty acid) nor linoleic or oleic acid had significant effects on the channel activity. Regarding the second possibility, Lundbæk and Andersen(1994) have recently demonstrated that lysophospholipids altered gramicidin channel function by altering the membrane deformation energy. Several lines of evidence argue against this mechanism being responsible for the effect of exposure to AA. First, a relatively high concentration (micromolar) of lipids is required to change the membrane deformation energy. In contrast, the effect of AA was produced by as little as 5 nM 20-HETE. Second, the effect of AA can be blocked by inhibition of the cytochrome P450 metabolic pathway. Finally, although the size and structure of 20-HETE are very similar to those of 19-HETE, addition of 19-HETE failed to mimic the effect of 20-HETE. Thus, our results strongly suggest that the effects of AA and its metabolites on the channel activity were not induced by alteration of membrane deformation energy.

AA plays an important role in the regulation of activity of ion channels in a variety of tissues (Kim and Duff, 1990; Kim, Lewis, Craziadei, Neer, Bar-Sagi, and Clapham, 1989; Kovalchuk, Miller, Sarantis, and Attwell, 1994; Ling et al., 1992; Piomelli, Volterra, Dale, Siegelbaum, Kandel, Schwartz, and Belardetti, 1987; Villarroel, 1993). AA exerts its effects by either direct or indirect action through mediation of its metabolites. For instance, AA has been shown to inhibit directly the activity of the apical K^+ channel in the cortical collecting duct (Wang et al., 1992), the outward-rectifying Cl^- channel in the airway epithelial cells (Anderson and Welsh, 1990) and the ATP-sensitive K^+ channel in isolated cardiac cells (Kim and Duff, 1990). On the other hand, lipoxygenase-dependent metabolites of AA regulate Na^+ channels in A6 cells, a cell line that has properties of CCD (Cantiello, Patenaude, Codina, Birnbaumer, and Ausiello, 1990). In cells other than renal tissue, AA-induced activation of the G-protein-coupled muscarinic gated K^+ channels in myocytes is mediated by 5-lipoxygenase metabolites (Kim et al., 1989). Lipoxygenase metabolites of AA play an important role in the modulation of activity of the serotonin (5-HT)-gated channel and are responsible for mediating presynaptic inhibition of Aplysia sensory neuron (Piomelli et al., 1987). Our data indicate that the inhibitory effect of AA on the activity of the 70-pS K^+ channel in the TAL was not attributable to AA per se, because the effect was completely abolished by 17-ODYA, an inhibitor of cytochrome P450 monooxygenase (Zou, Ma, Sui, Oritiz de Montellano, Clark, Masters, and Roman, 1994). In the rabbit kidney, the main metabolic pathway of AA in the TAL is cytochrome P450-dependent with 20-HETE and 20-COOH being the main metabolites (Carroll, Sala, Dunn, McGiff, and Murphy,

1991; Escalante et al., 1991). The P450 monooxygenase is also responsible for AA metabolism in the TAL of the rat kidney and 20-HETE is the main metabolite (Wang, unpublished observation). Two lines of evidence suggested that 20-HETE was a key mediator for the action of AA. First, 20-HETE can mimic the inhibitory effect of AA on the channel activity. Second, 20-HETE blocked the channel activity after inhibition of P450 monooxygenase with 17-ODYA.

20-HETE has been shown to inhibit the Na^+-K^+-ATPase in the proximal tubules (Schwartzman, Ferreri, Carroll, Songu-Mize, and McGiff, 1985) and the $Na^+/2Cl^-/K^+$ cotransport in the rabbit TAL (Escalante et al., 1991). Recently, it was demonstrated that 20-HETE plays a key role in the autoregulation of renal blood flow (Zou, Imig, Kaldunski, Ortiz de Montellano, Sui, and Roman, 1994). Our results also indicated that the effect of 20-HETE was specific, since addition of 100 nM 19-HETE, 17-HETE and 20-COOH failed to mimic the effects of 20-HETE. The inhibitory effects of 20-COOH on the $Na^+/2Cl^-/K^+$ cotransporter were as potent as those of 20-HETE in the rabbit TAL (Escalante et al., 1991). However, in the present study the inhibitory effect of 20-COOH on the activity of the 70-pS K^+ channel was much less significant than that of 20-HETE. Addition of 100 nM 20-COOH decreased the channel activity by only 20% whereas 10 nM 20-HETE completely blocked the channel activity. Because it was also observed that 20-COOH was not a major product of the P450-dependent metabolic pathway in the rat TAL (Wang, unpublished observations), the discrepancy may result from a species difference. Taken together, our results strongly suggest that 20-HETE is an important mediator of the AA effect on the apical K^+ channels. The mechanism by which 20-HETE exerts its effects is not understood. 20-HETE-induced inhibition of channel activity is not a fast block, since the main characteristic of a fast channel blockade is a decrease of the apparent channel current amplitude (Hille, 1992), which is not observed in the 20-HETE-induced blockade. On the other hand, it is still not clear whether 20-HETE-induced inhibition is a simple slow blockade. The mechanism of 20-HETE-induced blockade needs to be further explored.

The physiological role of P450 metabolites in the regulation of the activity of the apical K^+ channels is still not understood. However, it is of interest that inhibition of the P450 metabolic pathway stimulated the activity of the 70-pS K^+ channel, indicating that the channel activity was suppressed by P450 metabolites such as 20-HETE under physiological conditions and that the channel activity could be manipulated by the control of 20-HETE production. Interestingly, it was also observed that the channel activity in inside-out patches upon excision ($NP_o = 2.6 \pm 0.4$) was significantly higher than that in cell-attached patches ($NP_o = 1.5 \pm 0.2$), suggesting that channel activity was suppressed in cell-attached patches (data not shown). It is known that the rate-limited step for the production of 20-HETE is the AA release, which is regulated by several second messengers and hormones (Bonventre and Nemenoff, 1991). For instance, stimulation of phospholipase C, D, and phospholipase A_2 enhances the release of AA. In addition, increase in intracellular Ca^{2+} and the activity of protein kinase C stimulates the release of AA (Cockcroft, Nielson, and Stutchfield, 1991). Hormones such as vasopressin may increase the release of AA by stimulation of V1 receptor (Abramow, Beauwean, and Cogan, 1987). Angiotensin II (AII) has been shown to increase the release of AA and its metabolites in

the proximal tubules of rat kidney (Douglas and Hopfer, 1994). AII receptors have also been identified in the TAL (Mujais, Kauffman, and Kaz, 1986). Thus it is conceivable that stimulation of the AII receptor in the TAL may lead to an increase in AA release. AII and vasopressin have been shown to have significant effects on ion transport in the TAL (Hebert and Andreoli, 1984; Capasso, Unwin, Ciani, De Santo, De Tommaso, Russo, and Giebisch, 1994). Although only stimulatory effect of AII on Na^+ and HCO_3^- transport have been demonstrated by Capasso et al. (1994) AII may still have an inhibitory effect on ion transport in the TAL. For instance, it has been shown that effects of AII on Na^+ and HCO_3^- transport in the proximal tubule are biphasic (Wang and Chan, 1991). Indeed, our unpublished results show that AII at pM concentration blocked the activity of the 70-pS K^+ channel.

Since recycling K^+ across the apical membrane is one important step for the salt reabsorption in the TAL and 20-HETE is involved in determining the set-point for the activity of the 70-pS K^+ channel, 20-HETE could be an important element of the signal transduction pathways for mediating the effects of hormones, such as vasopressin and AII, on ion transport in the TAL.

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