EICOSANOIDS PLAY AN IMPORTANT role in the regulation of epithelial transport in the cortical collecting duct (CCD) which is responsible for the hormone-regulated Na absorption and K secretion (13, 15, 34). Two metabolic pathways of arachidonic acid, cytochrome P-450 (CYP) epoxygenase (31, 45) and cyclooxygenase (COX)I/COXII (36, 48), are present and involved in the regulation of the epithelial transport in the CCD. It has been reported that the metabolites of the CYP epoxygenase-dependent pathway of arachidonic acid inhibited Na absorption in the CCD (36) and that PGE2 regulated water and ion transport in the CCD (8). Three types of PGE2 receptors, EP1, EP3, EP4, are expressed in the CCD (8) and both EP1 and EP3 receptors have been shown to regulate the epithelial transport in the CCD. Activation of EP3 receptor suppresses cAMP production via stimulation of Gi protein and it is possibly responsible for facilitating dephosphorylation of aquaporin 2 (AQ P2) (49). Stimulation of EP1 receptor has been shown to raise the intracellular Ca2+ concentrations and PKC (1) and to be responsible for inhibition of Na transport in the CCD (16). PKC is known to regulate the apical small-conductance K (SK) channels in the CCD (20). Moreover, PKC has been shown to be involved in activation of mitogen-activated protein kinase (MAPK) in cultured mesangial cells and HEK293 cells (23, 40). Because activation of MAPK has been shown to inhibit both ROMK and big-conductance K (BK) channels (3, 26), it is expected that stimulation of EP1 receptor should inhibit ROMK and BK channels in the CCD. Furthermore, dietary K intake has been shown to affect PE2 production (2, 17). Thus it is possible that PGE2 may play a role in mediating the effect of K restriction on apical K channels in the CCD. Therefore, the aim of the present study is to test the hypothesis that PGE2 regulates apical K channels in the CCD by stimulation of PKC or MAPK.

METHODS

Preparation of CCDs. Pathogen-free Sprague-Dawley rats of either sex (5–6 wk) were used in experiments and were purchased from Taconic Farms (Germantown, NY). The protocol used in this study was reviewed and approved by the New York Medical College Institutional Animal Care and Use Committee. The animals were put on a high-K (HK; 10%, wt/wt), normal-K (NK; 1.1%), or a K-deficient (KD; <0.0001%; Harlan Teklad, Madison, WI) diet for 7 days before use. The rats were killed by cervical dislocation and the kidneys were removed immediately and cut into several thin slices (<1 mm) that were placed on an ice-cold Ringer solution until dissection. The dissection was carried out at room temperature and the single CCD was isolated and immobilized by placing the tube on a 5 × 5-mm coverglass coated with polylysine. The glass containing CCD was then 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 around the chamber. The CCD was cut open with a sharpened micropipette to expose the apical membrane.

Patch-clamp technique. Patch-clamp electrodes were made using a Narishige (P-81) puller with thick-wall glass capillaries (Degan, Minneapolis, MN) and had resistance of 4–6 MΩ when filled with 140 mM KCl. The pipettes were then fire-polished with a homemade polisher. An Axon 200A patch-clamp amplifier was used to record channel current. The current was low-pass filtered at 1 kHz by an eight pole Bessel filter (902LFP, Frequency Devices, Haverhill, MA) and digitized by an Axon interface (Digitida1200). Data were acquired by

* Y. Jin and Z. Wang contributed equally to this work.

Address for reprint requests and other correspondence: W.-H. Wang, Dept. of Pharmacology, New York Medical Univ., Valhalla, NY 10595 (e-mail: wenhui_wang@nymc.edu).

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an IBM-compatible Pentium computer (Gateway 2000) at a rate of 4 kHz and analyzed using the pClamp software system 6.04 (Axon Instruments, Burlingame, CA). Channel activity was defined as $NP_o$, which was calculated from data samples of 60-s duration in the steady state as follows:

$$NP_o = \sum (1_t + 2_t + \ldots + i_t)$$

where $i_t$ is the fractional open time spent at each of the observed current levels. The effect of PGE$_2$ on SK channels occurred within a 10-min period at which time we selected a representative 60-s-long recording to calculate channel activity. We used the CCD from rats on a NK diet unless it was indicated.

**Cell culture.** M-1 cells, a mouse collecting duct cell line, were obtained from the American Type Culture Collection (Manassas, VA) and grown in DMEM/F12 (1:1) medium with 2.5 mM l-glutamine, 15 mM HEPES, 0.5 mM sodium pyruvate, and 1.2 g/l sodium bicarbonate supplemented with 0.005 mM dexamethasone and 5% fetal bovine serum. M-1 cells were treated with PGE$_2$ for a different time period.

**Preparation of renal tissue for Western blot.** The renal cortex and the outer medulla were separated and suspended in RIPA buffer solution (1:8 ratio, wt/vol) containing 1× PBS, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 10 μl of PMSF (10 mg/ml stock solution in isopropanol). A cocktail of protease inhibitors (10 μl; Sigma, St. Louis, MO) was added per milliliter of buffer at the time of lysis. The samples were homogenized on ice for 15 min with a mortar and pestle and the suspension was incubated at 4°C for 1 h in the presence of DNase (5 μg/ml). After centrifugation, the supernatant was collected. We measured protein concentrations using a Bio-Rad D, protein assay kit.

**Western blot.** Proteins homogenized from renal tissue or M-1 cells were separated by electrophoresis on 8–10% SDS-polyacrylamide gels and transferred to Immuno-Blot PVDF membrane (Bio-Rad, Hercules, CA). The membrane was blocked with Odyssey blocking buffer for fluorescent Western blotting (Rockland, Gilbertsville, PA) and incubated with the primary antibody at 4°C for 12 h. The membrane was washed four times (each 5 min) with PBS containing 0.1% Tween 20 and followed by incubation with the secondary antibody for an additional 30 min. The membrane was then washed three times (10 min for each wash) with PBS and scanned by Odyssey infrared imaging system (LI-COR, Lincoln, NE) at wavelength of 680 or 800 nm.

**PGE$_2$ assay.** PGE$_2$ in the renal cortex and outer medulla was measured using a PGE$_2$ EIA kit (Cayman Chemical, Ann Arbor, MI). Briefly, homogenized tissue samples from renal cortex and outer medulla were mixed with aceton in 1:2 ratio for 5 min at room temperature. The mixture was then centrifuged at 1,500 g for 10 min to collect the supernatant followed by gently blowing the sample-containing tube with nitrogen gas to let acetone evaporate. Five hundred microliters of EIA buffer provided by the kit were added to the tube followed by vortex. After the sample was prepared, we followed the protocol provided by the manufacturer to construct a PGE$_2$ standard curve and measure the PGE$_2$ content in the sample (www.caymanchem.com/analysis).

**Experimental solutions and statistics.** The pipette solution contained (in mM) 140 KCl, 1.8 MgCl$_2$, and 10 HEPES (pH 7.4). The bath solution was composed (in mM) of 140 NaCl, 5 KCl, 1.8 CaCl$_2$, 1.8 MgCl$_2$, 5 glucose, and 10 HEPES (pH 7.4). SC-51089, sulprostone, calphostin C, GF109203X, SB202190, and PD98059 were purchased from Biomol (Plymouth Meeting, PA) and dissolved in either ethanol or DMSO solution. The final concentration of ethanol or DMSO was <0.1% and had no effect on channel activity. Data are shown as means ± SE. We used either Student’s t-test or one-way ANOVA to determine the significance between the two groups. Statistical significance was taken as $P < 0.05$.

**RESULTS.**

We first used the patch-clamp technique to examine the effect of PGE$_2$ on the ROMK-like SK channels in the CCD dissected from rats on a NK diet. Figure 1A is a channel recording demonstrating that application of 10 μM PGE$_2$ inhibited the SK channels. The inhibitory effect of PGE$_2$ was partially reversible because wash-out of PGE$_2$ partially restored the channel activity. Data summarized in Fig. 1B show that application of 10 μM PGE$_2$ significantly decreased $NP_o$ from 1.5 ± 0.13 to 0.63 ± 0.08 in rats on a NK diet ($n = 36$, $P < 0.001$). Application of PGE$_2$ also inhibited SK channels in rats on a HK diet (data not shown).

We also examined the effect of PGE$_2$ on the BK channel in the CCD from rats on a HK diet. The reason for using HK-adapted rats was that BK channel activity was higher in the CCD from rats on a HK diet than those on a NK diet. We observed coexistence of both BK channels and SK channels in some patches. However, those patches were not suitable to study the effect of PGE$_2$ on BK channels because either BK channel activity was too low or SK channel activity was too high such that it is impossible to analyze BK channel activity. Thus we selected patches in principal cells with high BK channel activity. Figure 2A is a channel recording showing that application of 10 μM PGE$_2$ inhibited BK channel activity in principal cells. Data summarized in Fig. 2B show that PGE$_2$ decreased $NP_o$ from 0.08 ± 0.01 to 0.04 ± 0.01 ($n = 4$). Moreover, the inhibitory effect of PGE$_2$ on BK channel was reversible (Fig. 2A). Thus PGE$_2$ inhibits both the SK and BK channels in the CCD.

We then explored the mechanism by which PGE$_2$ inhibits the SK channels. Because ROMK-like SK channels have been shown to be inhibited by PKC (50), we tested the possibility that PGE$_2$ could activate PKC via EP1 receptor which has been shown to be expressed in the CCD (8). Thus the effect of PGE$_2$ on the SK channels was examined in the presence of either calphostin C (100 nM) or GF-109203X (5 μM) to determine whether PKC mediated the effect of PGE$_2$. We first examined the effect of PKC inhibitors on channel activity and confirmed the previous report that inhibition of PKC did not significantly affect the SK channel activity (46). This suggests that PKC does not determine the basal activity of the SK channels or is not activated under control conditions. We then examined the effect of PGE$_2$ on SK channels in the CCD which has been pretreated with either calphostin C or GF-109203X for 20–30 min. Data summarized in Fig. 1B show that inhibition of PKC abolished the inhibitory effect of PGE$_2$ on the SK channels (calphostin C or GF-109203X, $NP_o = 1.35 ± 0.25$; PGE$_2$ + PKC inhibitors, $NP_o = 1.35 ± 0.25$; Fig. 1B). Thus this suggests that activation of PKC was responsible for the effect of PGE$_2$ on the SK channels.

Several studies showed that stimulation of PKC enhanced the activity of MAPK such as P38 and ERK (5, 14, 25) which have been shown to inhibit the SK channels (3). Thus we examined the role of P38 and ERK in mediating the effect of PGE$_2$ on the SK channels. Inhibition of both P38 and ERK not only increased the SK channel activity but also abolished the inhibitory effect of PGE$_2$ on the SK channels. Figure 1B summarizes the results showing that in the pres-
mediated by activation of PKC-MAPK pathway. Inhibition of ERK and P38 MAPK also abolished the effect of PGE2 on BK channels. Data summarized in Fig. 2B show that inhibition of ERK and P38 MAPK not only increased channel activity ($N_{Po} = 0.26 \pm 0.09$, $n = 3$) but also blocked the effect of PGE2 on BK channels ($N_{Po} = 0.25 \pm 0.09$, $n = 3$).

Because stimulation of EP1 receptor has been shown to activate PKC, we next examined the effect of PGE2 in the presence of SC-51089 (10 μM), a specific inhibitor of EP1 receptor (39). Data summarized in Fig. 3A show that inhibition of EP1 receptor completely abolished the inhibitory effect of 10 μM PGE2 on SK channels in the CCD from rats on NK and HK diet. The notion that PGE2-induced inhibition of SK channels in the CCD was the result of activation of EP1 receptor was further confirmed by experiments in which the effect of sulprostone, an agonist for EP1 and EP3 receptors (43), on SK channel activity was examined in the CCD. Figure 3B is a channel recording demonstrating that application of sulpro-

![Fig. 1.](image1.png)  

**Fig. 1.** A: channel recording demonstrating the effect of PGE2 (10 μM) on the ROMK-like small-conductance K (SK) channels in the cortical collecting duct (CCD). Top: trace is the time course of the experiment and the gap was 360 and 120 s, respectively. Three parts of the trace indicated by a shot bar and numbers were extended to show the fast time resolution. The channel close level is indicated by letter “C” and dotted lines and the pipette holding voltage was 0 mV. B: bar graph demonstrating that the effect of PGE2 on SK channel was abolished by blocking PKC or mitogen-activated protein kinase (MAPK). The effect of PGE2 on SK (n = 36) is shown in the left set of the bars. The middle and right sets of bars summarize the effect of PGE2 (10 μM) plus PKC inhibitors (100 nM calphostin C or 5 μM GF109203x; n = 6) and PGE2 + SB202190 (SB; 5 μM) + PD98059 (PD; 50 μM; n = 4), respectively. *Significant difference between the control and experimental group determined by Student’s t-test. #Channel activity in the CCD treated with SB+PD is significantly different from those without SB+PD (1-way ANOVA test).
tone (10 μM) inhibited the SK channels in the CCD of rats on NK and HK diet. Figure 3A shows that application of sulprostone decreased SK channel activity from 2.5 ± 0.33 to 1.16 ± 0.48 (n = 5). The channel activity shown in Fig. 3 is higher than that in Fig. 1 because we pooled data from rats on NK and HK diets. The inhibition of SK channels induced by sulprostone and PGE2 was the same in the CCD from rats on a NK or HK diet.

To further test the possibility that PGE2 inhibits the SK channels by stimulating P38 and ERK in the CCD, we used mouse CCD cells (M-1) to examine the effect of PGE2 on P38 and ERK phosphorylation because the response of ROMK channels in the mouse CCD to PGE2 is identical to that observed in the rat CCD (data not shown). Although M-1 cells, like any available CCD cells, do not have ROMK-dependent K secretion, M-1 cells possess some important biophysical properties of principal cells such as Na transport (24) and M-1 cells respond to PGE2 (37). Figure 4 is a Western blot demonstrating the dose-response curve of PGE2 effect on P38 (A) and ERK (B) phosphorylation. Application of 1, 5, and 10 μM PGE2 (5 min) increased the phosphorylation of P38 MAPK in M-1 cells by 20 ± 2, 50 ± 5 (P < 0.05), and 70 ± 8% (P < 0.01), respectively. PGE2 also stimulates the phosphorylation of ERK by 21 ± 4, 80 ± 10 (P < 0.05), and 100 ± 15% (P < 0.01), respectively. We next examined the time course of the effect of 10 μM PGE2 on the phosphorylation of P38 and ERK (see Fig. 6). From inspection of Fig. 5, it is apparent that 5-min treatment of M-1 cells with 10 μM PGE2 significantly increased phosphorylation of P38 MAPK and ERK by 70 ± 10% (P < 0.01; Fig. 6A) and by 85 ± 16% (P < 0.01; Fig. 6B), respectively. The PGE2-induced stimulation of P38 and ERK phosphorylation was diminished with a prolonged incubation and there was no significant difference between M-1 cells treated with PGE2 for 30 min and those without the treatment.

We next examined whether endogenous PGE2 was involved in determining the basal phosphorylation level of ERK and P38 by inhibition of COX. From inspection of Fig. 6, it is apparent that inhibition of endogenous COX with indomethacin (10 μM) did not affect the MAPK activity in M-1 cells. Moreover, the stimulatory effect of PGE2 on P38 and ERK phosphorylation was not affected by inhibiting COX because PGE2 still increased the phosphorylation of P38 (75 ± 10%, P < 0.05; Fig. 6A) and ERK (95 ± 10%, P < 0.05; Fig. 6B) in the presence of indomethacin (10 μM).

We then examined whether the effect of PGE2 on the phosphorylation of P38 and ERK was mediated by stimulation of PKC. We treated M-1 cells with 100 nM calphostin C, an inhibitor of PKC, and tested whether inhibition of PKC was able to block the effect of PGE2 on MAPK phosphorylation. Figure 7 is a Western blot showing that inhibition of PKC did not significantly affect the basal level of the phosphorylation of P38 (A) and ERK (B). However, application of calphostin C completely abolished the effect of PGE2 on the phosphorylation of P38 and ERK. Thus PKC possibly mediated the stimulatory effect of PGE2 on MAPK phosphorylation in M-1 cells.

Since low-K intake has been shown to increase the phosphorylation of P38 and ERK (3), we speculate that PGE2 may be involved in mediating the effect of K restriction on P38 and ERK phosphorylation. This hypothesis was tested by examining the expression of COXII and PGE2 production in the renal cortex and outer medulla. Figure 8A is a Western blot demonstrating that low-K intake significantly increased the expression of COXII by 96 ± 10% (P < 0.05) compared with those on a NK diet (1.1%). In contrast, HK intake significantly decreased COXII expression by 90 ± 20% (n = 3) in the renal cortex and outer medulla (a mixture; Fig. 8A, bottom). Also, Fig. 8B shows that low-K intake significantly increased PGE2...
production from 1,203 ± 137 to 3,609 ± 460 pg/mg protein ($P < 0.05$) in renal cortex and outer medulla. Thus K restriction increased both COXII expression and PGE2 production which is expected to stimulate MAPK phosphorylation.

DISCUSSION

COX-dependent metabolites of arachidonic acid play an important role in the regulation of renal blood flow, renin secretion, and epithelial transport in the kidney (7, 18). PGE2 has been demonstrated to inhibit NaCl absorption in the thick ascending limb (TAL) (22, 41) and the inhibitory effect of PGE2 was the result of antagonizing the effect of vasopressin on cAMP production (10). We previously observed that PGE2 inhibited the apical 70-pS K channels in the rat TAL (27). Since K recycling is essential for maintaining the function of Na-K-Cl cotransporter, PGE2-induced inhibition of apical K channels should lead to inhibition of NaCl transport in the TAL. Also, a large body of evidence indicates that the COX-dependent metabolites of arachidonic acid play a role in the regulation of the transport function of the CCD. PGE2 has also been shown to inhibit Na-K-ATPase (44) and stimulate Cl secretion in mouse CCD cells (37). The importance of PGE2 in the regulation of the epithelial transport in the CCD is also supported by the finding that the prostaglandin transporter

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**Fig. 4.** Western blot showing the effect of PGE2 on the phosphorylation of P38 (A) and ERK (B) in M-1 cells treated with 1, 5, and 10 μM PGE2 for 5 min ($n = 4$). The total level of MAPK expression in M-1 cells is shown in the middle and bar graphs summarizing the corresponding results are demonstrated on the bottom. If data are significantly different from the control value (no PGE2), the $P$ value is indicated.

**Fig. 5.** Western blot showing the time course of PGE2 effect on the phosphorylation of P38 (A) and ERK (B) in M-1 cells treated with 10 μM PGE2 for 5, 10, 15, 30 min ($n = 3$). The total level of MAPK expression in M-1 cells is shown in the middle and bar graphs summarizing the corresponding results are demonstrated on the bottom. If data are significantly different from the control (0 min), the $P$ value is indicated.
which is responsible for secretion or reuptake of PGE₂ is highly expressed in the CCD (32). Thus PGE₂ level in the CCD is controlled not only by COXⅠ and COXⅡ activity but also by the prostaglandin transporter activity. COX-dependent metabolic pathway was responsible for 5,6-EET-mediated inhibition of Na transport in the CCD (16, 19, 21, 42). The present observation that PGE₂ inhibited ROMK-like SK channels and BK channels has further indicated that PGE₂ is also involved in the regulation of the K secretion in the CCD because the ROMK-like SK channels (12, 20, 38) and the BK channels (4, 31).

Fig. 6. Western blot showing the effect of PGE₂ on the phosphorylation of P38 (A) and ERK (B) in M-1 cells treated with 10 μM PGE₂ for 5 min in the presence or absence of indomethacin (5 μM). The total level of MAPK expression in M-1 cells is shown in the middle and bar graphs summarizing the corresponding results are demonstrated on the bottom (n = 4). If data are significantly different from the control (no PGE₂ treatment), the P value is indicated.

Fig. 7. Western blot showing the effect of PGE₂ on the phosphorylation of P38 (A) and ERK (B) in M-1 cells treated with 10 μM PGE₂ for 5 min in the presence or absence of calphostin C (100 nM). The total level of MAPK expression in M-1 cells is shown in the middle and bar graphs summarizing the corresponding results are demonstrated on the bottom (n = 4). If data are significantly different from the control (no PGE₂ treatment), the P value is indicated.
abolished the effect of PGE2. Moreover, we also observed that inhibi-
tion of PKC or application of EP1 receptor antagonist is closely related to the stimulation of EP1 receptor because either low concentrations of PGE2 (1 μM) inhibit SK channels (Wang W-H, unpublished results).

Three types of PGE2 receptors, EP1, EP3, and EP4, have been shown to be expressed in the CCD (9). Stimulation of the EP4 receptor located in the apical membrane has been reported to increase the water permeability by increasing cAMP in the CCD (35). However, it is unlikely that the inhibitory effect of PGE2 on apical K channels was mediated by EP4 receptor because an increase in cAMP is expected to stimulate SK channel activity (30). Stimulation of the EP3 receptor is known to decrease cAMP levels and accordingly suppress PKA activity. However, the observation that inhibition of PKA did not affect the basal activity of SK channels in the CCD (28) excludes the possibility that the EP3 receptor is responsible for mediating the effect of PGE2 on SK. Thus it is most likely that the EP1 receptor was responsible for mediating the inhibitory effect of PGE2 on SK channels. This view was supported by three lines of evidence. 1) Inhibition of PKC abolished the PGE2-induced inhibition of SK channels. 2) Application of sulprostone mimics the effect of PGE2 and inhibits ROMK channels. 3) SC-51089 blocked the effect of PGE2 on ROMK channels in the CCD. It is possible that PKC is an upstream signal molecule responsible for the PGE2-induced activation of P38 and ERK which further inhibit the SK channels. This notion is also supported by two observations. 1) Inhibition of PKC abolished the effect of PGE2-induced stimulation of P38 and ERK phosphorylation in M-1 cells. 2) Blockade of P38 and ERK MAPK mimicked the effect of inhibiting PKC and abolished the inhibitory effect of PGE2 on the SK channels. Thus the present study suggests that PGE2-induced inhibition of the SK channels is the result of stimulating the PKC-MAPK pathway.

The previous study showed that P38 and ERK MAPK play an important role in the regulation of the SK channels (3). Moreover, the finding that inhibition of P38 and ERK increased the activity of SK channel activity suggests that P38 and ERK MAPK are involved in determining the basal activity of SK channels. In contrast to P38 and ERK MAPK, inhibition of PKC did not significantly increase the basal level of SK channel activity as reported previously (46). This suggests that PKC is not activated under control conditions or that PKC does not regulate the basal activity of the SK channels. The activity of P38 and ERK MAPK is regulated by dietary K intake such that low-K intake stimulates while HK intake suppresses the phosphorylation of P38 and ERK MAPK. The effect of K intake on ERK and P38 MAPK phosphorylation is at least partially mediated by superoxide and related products because suppressing superoxide levels with tempol abolished the effect of low-K intake on P38 and ERK phosphorylation (3). It is also well-established that stimulation of PKC could activate MAPK in a variety of cells (5, 14, 25). Although the mechanism by which PKC activates MAPK is not completely understood, PKC could activate NADPH oxidase by phosphorylation of p47phox (6) and increase the superoxide generation that stimulates the phosphorylation of ERK and P38 MAPK (3).

We also showed that PGE2 inhibits BK channel activity in principal cells. The role of BK channels in mediating renal K secretion has been recently established (4, 47). Because BK channel activity is also inhibited by P38 and ERK MAPK (26), it is conceivable that PGE2 inhibits the BK channels possibly through PKC-MAPK pathways. This view is supported by the observation that inhibition of P38 and ERK MAPK not only increased the BK channel activity but also abolished the effect of PGE2 on BK channels in the CCD. The PGE2-mediated regulation of SK and BK channels may play a role in suppression of renal K secretion during K restriction because low-K intake stimulates while high-K intake suppresses the activity of SK channel (3). However, it is unlikely that the inhibitory effect of PGE2 on apical K channels was mediated by EP4 receptor because an increase in cAMP is expected to stimulate SK channel activity (30). Stimulation of the EP3 receptor is known to decrease cAMP levels and accordingly suppress PKA activity. However, the observation that inhibition of PKA did not affect the basal activity of SK channels in the CCD (28) excludes the possibility that the EP3 receptor is responsible for mediating the effect of PGE2 on SK. Thus it is most likely that the EP1 receptor was responsible for mediating the inhibitory effect of PGE2 on SK channels. This view was supported by three lines of evidence. 1) Inhibition of PKC abolished the PGE2-induced inhibition of SK channels. 2) Application of sulprostone mimics the effect of PGE2 and inhibits ROMK channels. 3) SC-51089 blocked the effect of PGE2 on ROMK channels in the CCD. It is possible that PKC is an upstream signal molecule responsible for the PGE2-induced activation of P38 and ERK which further inhibit the SK channels. This notion is also supported by two observations. 1) Inhibition of PKC abolished the effect of PGE2-induced stimulation of P38 and ERK phosphorylation in M-1 cells. 2) Blockade of P38 and ERK MAPK mimicked the effect of inhibiting PKC and abolished the inhibitory effect of PGE2 on the SK channels. Thus the present study suggests that PGE2-induced inhibition of the SK channels is the result of stimulating the PKC-MAPK pathway.

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effect on COXII expression and PGE\textsubscript{2} generation. Thus decreased PGE\textsubscript{2} levels may be partially responsible for augmentation of SK and BK channel activity in HK-adapted rats. In this regard, it has also been reported that BK channels are involved in renal K secretion only in animals on a HK intake (4).

Dietary K intake has been shown to affect PGE\textsubscript{2} synthesis in the kidney. However, the effect of K intake on renal PGE\textsubscript{2} levels is varied: low-K intake for 3 wk has been shown to decrease urinary PGE\textsubscript{2} excretion in rabbit kidney (2) while the K restriction for 9 days has been reported to increase urinary PGE\textsubscript{2} excretion in the rat kidney (17). Thus the effect of K restriction on PGE\textsubscript{2} synthesis depends on the animal species and duration of K depletion. The mechanism by which low-K intake stimulates COXII expression and PGE\textsubscript{2} production is not known. Low-K intake has been shown to increase renin and angiotensin system activity (33). Also, renin has been reported to increase PGE\textsubscript{2} biosynthesis in human amnion cells (29).

Angiotensin II has been demonstrated to increase PGE\textsubscript{2} generation of COXII in the mouse kidney (51), acute application of angiotensin II stimulation of COXII expression and PGE\textsubscript{2} production. Application of renin has been reported to increase PGE\textsubscript{2} biosynthesis in human amnion cells (29). Although chronic angiotensin II infusion inhibits the expression of COXII in the mouse kidney (51), acute application of angiotensin II has been demonstrated to increase PGE\textsubscript{2} generation in the medullary TAL (11). Thus further experiments are needed to explore the mechanism by which low-K intake stimulates COXII expression and PGE\textsubscript{2} production.

In summary, we demonstrated that low-K intake stimulates the expression of COXII and PGE\textsubscript{2} generation. Application of PGE\textsubscript{2} inhibits SK and BK channels in the CCD and stimulates the phosphorylation of P38 and ERK MAPK. The PGE\textsubscript{2}-induced inhibition of SK channels is mediated by activation of EP1 receptor and the PKC-MAPK pathway.

**GRANTS**

This work is supported by National Institutes of Health Grant DK-47402 (W.-H. Wang) and HL-34100 (W.-H. Wang). Dr. B. F. Yang is supported by National Science Foundation of China 30430780.

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