K Depletion Enhances the Extracellular Ca^{2+}-induced Inhibition of the Apical K Channels in the mTAL of Rat Kidney

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ABSTRACT We have shown previously that raising extracellular Ca^{2+} inhibited the apical 70-pS K channel in the thick ascending limb (TAL; Wang, W.H., M. Lu, and S.C. Hebert. 1996. *Am. J. Physiol.* 270:C103–C111). We now used the patch-clamp technique to study the effect of increasing the extracellular Ca^{2+} on the 70-pS K channel in the mTAL from rats on a different K diet. Increasing the extracellular Ca^{2+} from 10 μM to 0.5, 1, and to 1.5 mM in the mTAL from rats on a K-deficient (KD) diet inhibited the channel activity by 30, 65, and 90%, respectively. In contrast, raising the extracellular Ca^{2+} to 1.5 mM had no significant effect on channel activity in the mTAL from animals on a high K (HK) diet and further increasing the extracellular Ca^{2+} to 2.5, 3.5, and 5.5 mM decreased the channel activity by 29, 55, and 90%, respectively. Inhibition of the cytochrome P450 monooxygenase completely abolished the effect of the extracellular Ca^{2+} on channel activity in the mTAL from rats on a different K diet. In contrast, blocking cyclooxygenase did not significantly alter the responsiveness of the 70-pS K channel to the extracellular Ca^{2+}. Moreover, addition of sodium nitropruside, a nitric oxide (NO) donor, not only increased the channel activity, but also blunted the inhibitory effect of the extracellular Ca^{2+} on the 70-pS K channel and decreased 20-hydroxyeicosatetraenoic acid (20-HETE) concentration in the mTAL from rats on a KD diet. In contrast, inhibiting NOS with L-NAME enhanced the inhibitory effect of the extracellular Ca^{2+} on the channel activity and increased 20-HETE concentration in the mTAL from rats on a high K diet. Western blot has further shown that the expression of inducible NO synthase (iNOS) is significantly higher in the renal medulla from rats on an HK diet than that on a KD diet. Also, addition of S-nitroso-*N*-acetylpenicillamine abolished the inhibitory effect of arachidonic acid on channel activity in the mTAL, whereas it did not block the inhibitory effect of 20-HETE. We conclude that a low dietary K intake increases the sensitivity of the 70-pS K channel to the extracellular Ca^{2+}, and that a decrease in NOS activity is involved in enhancing the inhibitory effect of the extracellular Ca^{2+} on channel activity in the mTAL during K depletion.

KEY WORDS: 20-hydroxyeicosatetraenoic acid • iNOS • cytochrome P450 • calcium-sensing receptor • K recycling

INTRODUCTION

We have previously reported that raising the extracellular Ca^{2+} inhibited the apical 70-pS K channel in the thick ascending limb (TAL;* Wang et al., 1996). The effect of increasing the extracellular Ca^{2+} is most likely mediated by stimulating the Ca^{2+}-sensing receptor (CaR) in the mTAL. Moreover, the effect of the extracellular Ca^{2+} on channel activity was induced by stimulating the cytochrome P450 metabolic pathway of arachidonic acid (AA) because inhibiting ω-hydroxylation of cytochrome P450 abolished the effect of the extracellular Ca^{2+}. Furthermore, we have demonstrated that increasing the extracellular Ca^{2+} enhanced the formation of 20-hydroxyeicosatetraenoic acid (20-HETE), a major metabolite of cytochrome P450 ω-hydroxylation of AA in the mTAL (Carroll et al., 1991; Wang et al., 1997a). This suggests that 20-HETE may be responsible for mediating the effect of the extracellular Ca^{2+}.

We recently reported that a dietary K intake affects 20-HETE production: a low K intake stimulates, whereas a high K intake suppresses the 20-HETE generation (Gu et al., 2001). Since 20-HETE is the mediator for the effect of increasing the extracellular Ca^{2+}, it is possible that the response of the apical 70-pS K channel to the extracellular Ca^{2+} differs in mTAL harvested from animals on a high K (HK) or a K-deficient (KD) diet. In addition, NOS has been shown to be expressed in the renal mTAL (Ahn et al., 1994; Mohaupt et al., 1994; Shin et al., 1999). Our previous experiments illustrated that NO stimulates the 70-pS K channel in the mTAL by a cGMP-dependent protein kinase (Lu et al., 1998). Moreover, as NO has been demonstrated to inhibit the cytochrome P450 ω-hydroxylation (Sun et al., 1998; Ito and Roman, 1999; Oyekan et al., 1999), it is conceivable that NO may be involved in modifying the effect of increasing the extracellular Ca^{2+} by either increasing cGMP or suppressing cytochrome P450 metab-

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*Abbreviations used in this paper: AA, arachidonic acid; CaR, Ca^{2+}-sensing receptor; HK, high K; iNOS, inducible NO synthase; KD, K-deficient; NO, nitric oxide; P_o, open probability; SNP, sodium nitropruside; TAL, thick ascending limb.

olism of AA. Therefore, the aim of this investigation is to explore the role of cytochrome P450 metabolites of AA and NO in modulating the effect of increasing the extracellular Ca^{2+} on channel activity in the rat mTAL.

MATERIALS AND METHODS

Preparation of mTAL

Pathogen-free Sprague-Dawley rats (Taconic Farms) were used in the experiments. Animals were kept on different K diets including a high K diet (10%, wt/wt), a normal K diet, and a K-deficient (KD) diet (<0.001%; Harlan Teklad) for 7 d before use. Previous studies demonstrated that plasma K concentration in rats on a KD diet was lower (2.9 mEq/liter), whereas plasma K concentration in rats on an HK diet was higher (5.3 mEq/liter) than that in rats on a normal K diet (4.1 mEq/liter; Wei et al., 2001). The method for preparation of the mTAL has been described previously. The tubules were placed on a 5 × 5-mm coverglass coated with Cell-Tak (Collaborative Research). The cover glass was transferred to a chamber mounted on an inverted microscope (Nikon Inc.), and the tubules were superfused with bath solution containing the following (in mM): 140 NaCl, 5 KCl, 1.8 $MgCl_2$, 1.8 $CaCl_2$, 5 glucose, and 10 HEPES, pH 7.4. We used a sharpened pipette to open the mTAL to gain access to the apical membranes.

Western Blot

Protein samples extracted from the kidney cortex and medulla were separated by electrophoresis on 10% SDS–polyacrylamide gels and transferred to nitrocellulose membranes. The membranes were blocked with 10% nonfat dry milk in Tris-buffered saline (TBS), rinsed, and washed with 1% milk in Tween-TBS. The iNOS and nNOS antibodies were purchased from Transduction Laboratories and were diluted at 1:500. The protein concentration used for immunoblot was 50 μg. The proteins of iNOS and nNOS were detected and quantitatively analyzed by fluorescence phosphorimaging.

Measurement of 20-HETE

Freshly isolated mTALs (100–200 μg protein) from rats on a KD diet or on an HK diet were divided into control groups (treated with vehicle) or experimental groups (treated with 20 μM SNP for the tubules from KD animals or with 0.2 mM L-NAME for the tubules from HK animals). The tubules were suspended in 100 μl bath solution containing 5 μM L-arginine and incubated for 10 min at 37°C. To measure 20-HETE concentrations in the presence of different extracellular Ca^{2+} concentrations, the tubules obtained from rats on a different K diet (normal K, HK, and KD) were incubated for 15 min in the presence of 10 μM, 250 μM, 0.5 mM, and 2.5 mM extracellular Ca^{2+}, respectively. The incubation was terminated by decreasing temperature to 0°C with ice. As an internal standard, 1 ng of deuterated [14,15-$^2H_2$]-20-HETE was mixed with the tubules. The tissue was spun down and the supernatant was collected and evaporated. 1 ml distilled water was added to the tube containing the tubules and lipids were extracted with ethyl acetate acidified with formic acid, pH 3.5. The extract was dissolved in 100 μl methanol and separated by reverse-phase HPLC using a gradient of acetonitrile in water (50–100% in 20 min) at a flow rate of 1 ml/min. The 20-HETE fraction was dried, resuspended in 100 μl acetonitrile, and converted to pentaflurobenzyl (PFB) ester by adding 10 μl PFB bromide and 10 μl of *N,N*-diisopropylethylamine. The mixture was incubated at room temperature for 30 min. The sample was evaporated under nitrogen and further incubated with 80 μl *N,O*-bistrimethylsilyl-trifluoroacetamide for 30 min to collect the PFB ester, trimethylsilyl (TMS) ether derivative of 20-HETE. The sample was dried and dissolved in 50 μl of isooctane for GC/MS analysis (HP5989A mass spectrometer interfaced with an HP 5890 gas chromatograph). The samples were injected into a 10 × 0.25-mm DB-1 capillary column with 0.25-μm film thickness (J&W Scientific). Helium was used as the carrier gas to raise the temperature to 180–300°C with 25°/min of step increase. A selected ion monitoring negative chemical ionization was used to record ion abundance at m/z 391 and m/z 393, which corresponded to the endogenous and deuterated derivatized 20-HETE (PFB ester TMS ether), respectively. The concentration of total 20-HETE in the purified biological samples was calculated by comparison of the ion abundance ratio (m/z 391/393) versus a standard curve of 20-HETE-PFB-TMS/[$^2H_2$]-20-HETE-PFB-TMS molar ratio constructed by NCl^-GC/MS analysis.

Patch-clamp Technique

Electrodes were pulled with a vertical pipette puller (model PP83; Narishige) and had resistances of 4–6 MΩ when filled with 140 mM NaCl. The channel current recorded by an Axon200A patch-clamp amplifier was low-pass filtered at 1 KHz using an 8-pole Bessel filter (model 902LPF; Frequency Devices). The current was digitized by an Axon interface (Digitada1200) and collected by an IBM-compatible Pentium computer at a rate of 4 kHz and analyzed using the pClamp software system 6.04 (Axon Instruments). Channel activity was defined as NP_o, a product of channel open probability (P_o) and channel number (N). The NP_o was calculated from data samples of 60 s duration in the steady state as follows:

$$NP_o = \Sigma(1t_1 + 2t_2 + \ldots it_i),$$

where t_i is the fractional open time spent at each of the observed current levels. Since three types of K channels have been identified in the mTAL (Guggino et al., 1987; Taniguchi and Guggino, 1989; Bleich et al., 1990; Wang et al., 1997b), we measured the channel current at three different membrane potentials in each patch to estimate the conductance of the K channel in the patch.

Solution and Statistics

The pipette solution was composed of the following (in mM): 140 KCl, 1.8 Mg_2Cl, and 5 HEPES, pH 7.4. Indomethacin, L-NAME, and SNP were obtained from Sigma-Aldrich and SNAP was purchased from Biomol. DDMS was synthesized in Dr. Falck's laboratory at Southwest Medical Center of University of Texas and dissolved in DMSO. The final concentration of DMSO was <0.1% and had no effect on channel activity. During experiments, the bath solution was occasionally switched to a media containing 10 μM Ca^{2+} as a control solution. The data are presented as mean ± SEM. We used paired and unpaired *t* tests to determine the statistical significance. If the P value is less than 0.05, then the difference is considered to be significant.

RESULTS

We first examined the effect of increasing the extracellular Ca^{2+} on the apical K channel activity in the mTAL harvested from rats on a KD diet. Since raising the extracellular Ca^{2+} inhibited only the 70-pS K channel but not the 30-pS K channel (Wang et al., 1996), we focused our studies on exploring the effect of the extracellular Ca^{2+} on the 70-pS K channel. Fig. 1 A is a recording showing the effect of raising the extracellular Ca^{2+} on the 70-pS K channel in a cell-attached patch. Increasing the extracellular Ca^{2+} from 10 μM to 0.5, 1, and to 1.5

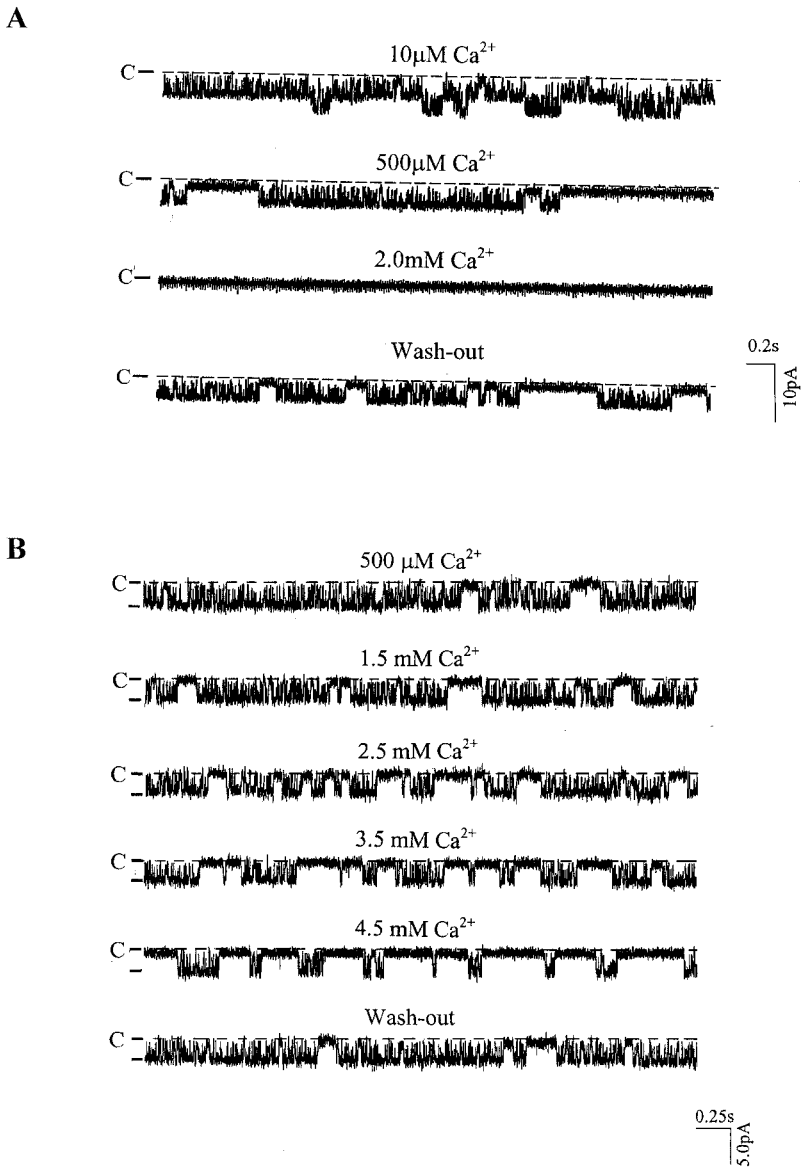

FIGURE 1. (A) The effect of increasing the extracellular Ca^{2+} on the activity of the 70-pS K channel in a cell-attached patch of the mTAL harvested from rats on a K-deficient diet. The mTAL was bathed in a 10-μM free Ca^{2+}-containing bath solution under control conditions. (B) The effect of the external Ca^{2+} on the activity of the 70-pS K channel in a cell-attached patch of the mTAL harvested from rats on a high K diet. The mTAL was bathed in a 500-μM Ca^{2+}-containing bath solution under control conditions. The channel closed states are indicated by "C," and the holding potential was 0 mV.

mM reduced NP_o by 30 ± 2%, 65 ± 5%, and 90 ± 9% (n = 10 patches), respectively. Fig. 2 is a dose–response curve showing the effect of increasing the extracellular Ca^{2+} on channel activity. It is estimated that K_i value, a concentration of the extracellular Ca^{2+} required for inhibiting the channel activity by 50%, is ~0.9 mM in the mTAL obtained from rats on a KD diet. This value is significantly lower than that (1.8 mM) observed in the mTAL from rats on a normal diet (Fig. 2).

We next tested the effect of the extracellular Ca^{2+} on channel activity in the mTAL from rats on an HK diet. Fig. 1 B is a typical recording demonstrating the effect of increasing the extracellular Ca^{2+} on the 70-pS K channel. It is apparent that raising the extracellular Ca^{2+} to 1.5 mM, which almost completely inhibited the channel activity in the tubule from rats on a KD diet, had no effect on the 70-pS K channel in the mTAL from rats on an HK diet. Further increasing extracellular Ca^{2+} to 2.5,

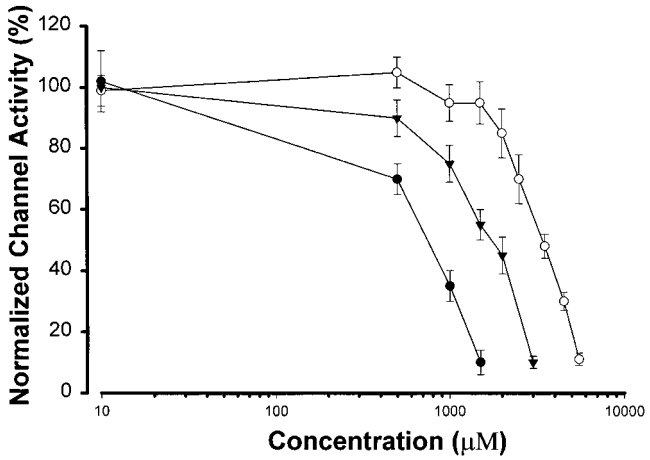

FIGURE 2. The dose–response curve of the 70-pS K channel to changing the extracellular Ca^{2+} concentrations in the mTAL from rats on a K-deficient diet (closed circle), on a normal diet (closed triangle) and on a high-K diet (open circle), respectively.

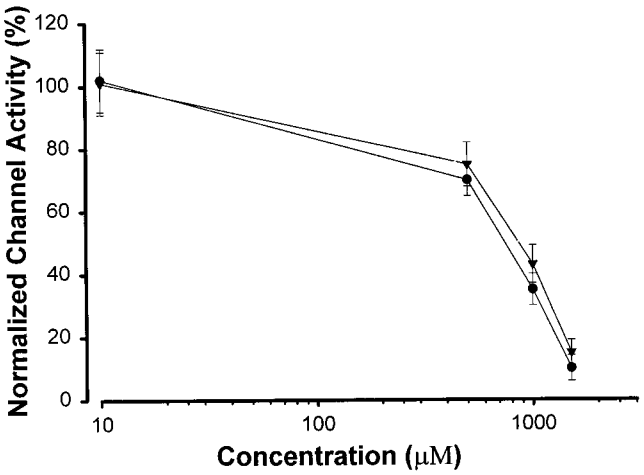

FIGURE 3. The effect of the extracellular Ca^{2+} on the 70-pS K channel in the mTAL from rats on a K-deficient diet in the presence of 5 μM indomethacin (closed triangle) and in the absence of indomethacin (closed circle).

3.5, 4.5, and to 5.5 mM decreased NP_o by 29 ± 2%, 55 ± 5%, 70 ± 6%, and 90 ± 6% ($n = 9$), respectively. From inspection of Fig. 2, it is clear that the dose–response curve of the extracellular Ca^{2+} effect shifts significantly to the right and K_i is ~3.4 mM. This suggests that the responsiveness of the 70-pS K channel to the extracellular Ca^{2+} diminished in the mTAL from rats on an HK diet. The K depletion has been shown to increase PGE_2 and 20-HETE generation (Rutecki et al., 1982; Gullner et al., 1983; Gu et al., 2001), agents which have been shown to inhibit the 70-pS K channel in the mTAL (Wang and Lu, 1995; Liu et al., 2000). Therefore, we examined the effect of the extracellular Ca^{2+} in the presence of indomethacin or DDMS to determine whether increasing PGE_2 or 20-HETE was responsible for magnifying the sensitivity of the K channel to the extracellular Ca^{2+}. We confirmed the preceding observation (Gu et al., 2001) that indomethacin increased the channel activity slightly but significantly (unpublished data). However, inhibiting cyclooxygenase did not significantly alter the responsiveness of the 70-pS K channel to the extracellular Ca^{2+}. Fig. 3 summarizes the results of eight experiments in which the effect of raising the extracellular Ca^{2+} was determined in the presence of 5 μM indomethacin in the mTAL from rats on a KD diet. Clearly, the sensitivity of the channel activity to the extracellular Ca^{2+} was almost identical in the presence of indomethacin or in the absence of the cyclooxygenase inhibitor. In contrast, inhibiting cytochrome P450 monooxygenase abolished the effect of the extracellular Ca^{2+} on channel activity in the mTAL from rats on a KD diet. Fig. 4 A is a recording demonstrating that in the presence of 5 μM DDMS, an agent which inhibits P450 ω-hydroxylation of AA (Wang et al., 1998; Nguyen et al., 1999), increasing extracellular Ca^{2+} to 5 mM did not inhibit the channel significantly in the mTAL from rats on a KD diet. Fig. 4 B summarizes the results of four experiments showing that the inhibitory effect of the extracellular Ca^{2+} on channel activity was absent in the tubules treated with DDMS from rats on a KD diet. In the presence of DDMS, the effect of the extracellular Ca^{2+} (5 mM) on channel activity also was blocked in the mTAL from rats on a normal K and an HK diet (unpublished data).

Therefore, our results indicate that an increase in 20-HETE production is responsible for enhancing the response of the apical 70-pS K channel to the extracellular Ca^{2+}. This notion also is supported by experiments in which the relationship between the extracellular Ca^{2+} and 20-HETE concentration was determined (Fig. 5). It is apparent that an increase in extracellular Ca^{2+} from 10 μM to 0.5 mM results in a sharp increase in 20-HETE production in the mTAL from rats on a KD diet. In contrast, the same increase in the extracellular Ca^{2+} concentration has no significant effect on the 20-HETE production in the mTAL from rats on a normal K or on an HK diet. To explore the possible mechanism by which 20-HETE production increases in the mTAL from rats on a KD diet, we extended our study to examine the interaction of NO with the cytochrome P450 metabolism of AA. The rationale for this investigation is that several studies had suggested that NO inhibited the cytochrome P450-dependent ω-hydroxylation of AA and, accordingly, diminished 20-HETE generation (Sun et al., 1998; Ito and Roman, 1999; Oyekan et al., 1999). Moreover, iNOS and nNOS have been shown to be expressed in the mTAL (Ahn et al., 1994; Mohaupt et al., 1994; Shin et al., 1999). We first examined whether a dietary K intake affects the expression of iNOS in the kidney (Fig. 6). It is apparent that the expression of iNOS in renal outer medulla increased from rats on an HK diet by 80 ± 10% ($n = 4$ rats), whereas it decreased from rats on a KD diet by 40 ± 6% in comparison to those on a normal K diet. In contrast, the expression of nNOS is the same in the renal medulla from rats on an HK, a KD, or a normal K diet (unpublished data).

After establishing that the renal expression of iNOS decreased in rats on a KD diet, we investigated the possibility of whether a diminished NO production was partially responsible for increasing the sensitivity of the 70-pS K channel to the extracellular Ca^{2+}. Fig. 7 A is a representative recording showing the effect of raising the extracellular Ca^{2+} on channel activity in the presence of 20 μM SNP, a donor of NO, in the mTAL from rats on a KD diet. Clearly, application of SNP not only increased the activity of the 70-pS K channel, but also attenuated the inhibitory effect of the extracellular Ca^{2+} because 2.5 mM Ca^{2+} was required to reduce the channel activity by 70 ± 6% in contrast to 1 mM in the absence of SNP (Fig. 7 B). Fig. 7 B summarizes the results of 20 experiments demonstrating that in the presence of SNP,

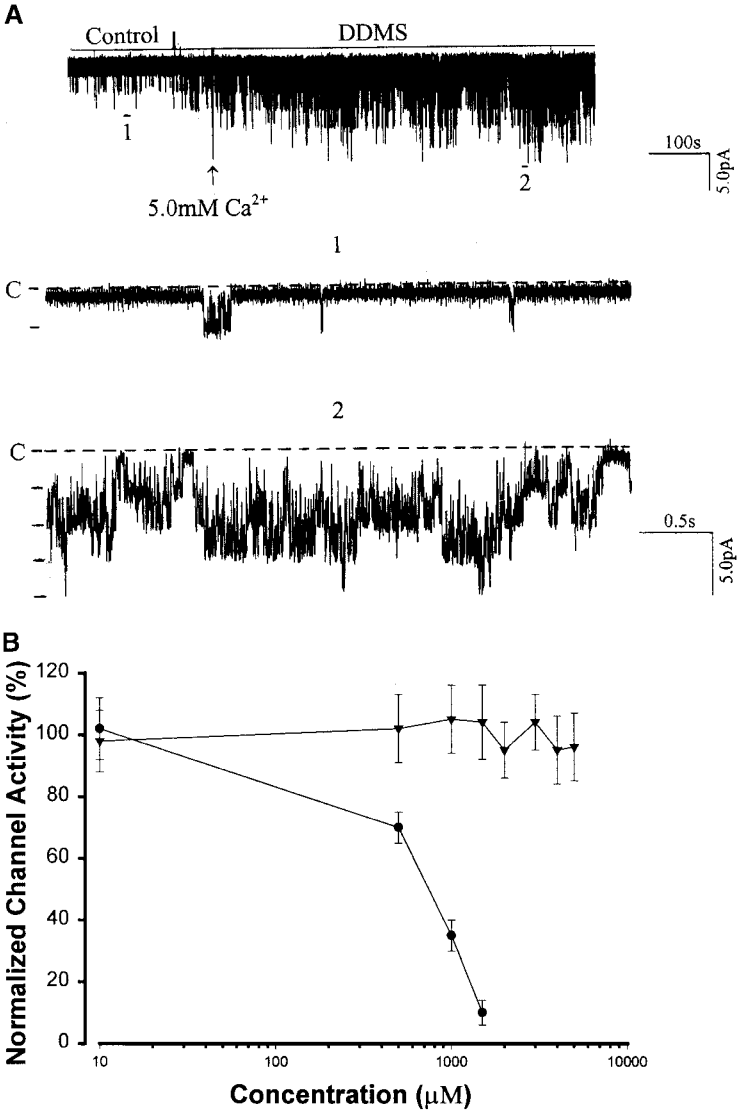

FIGURE 4. (A) A channel recording showing the effect of 5 mM external Ca^{2+} on the activity of the 70-pS K channel in the presence of 5 μM DDMS. The top trace shows the time course of experiments, and the two parts of the data indicated by numbers are extended to show the fast time resolution. The channel closed state is indicated by "C," and the holding potential was 0 mV. The extracellular Ca^{2+} under the control conditions was 10 μM (B). The dose–response curve of the 70-pS K channel to changes in the external Ca^{2+} concentrations in the mTAL from rats on a K-deficient diet in the presence of DDMS (closed triangle) and in the absence of DDMS (closed circle). The steady state of channel activity after application of DDMS was taken as a control and used to compare the channel activity observed in the presence of a given concentration of Ca^{2+}.

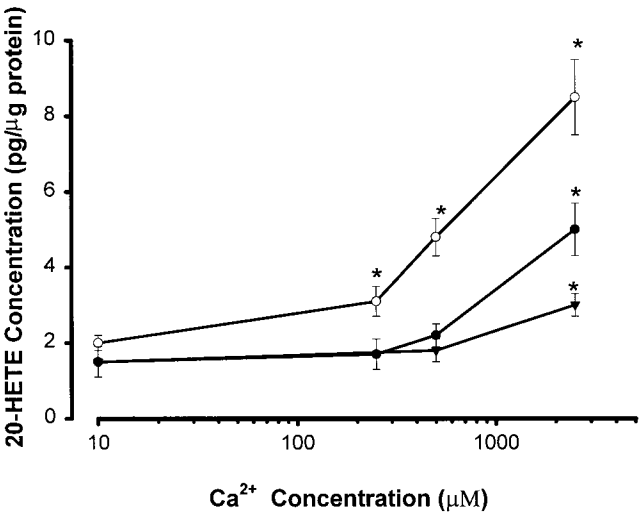

FIGURE 5. A dose–response curve between 20-HETE concentrations and extracellular Ca^{2+} in the mTAL from rats on different K diets including high K (closed triangle), normal K (closed circle), and K-deficient diet (open circle).

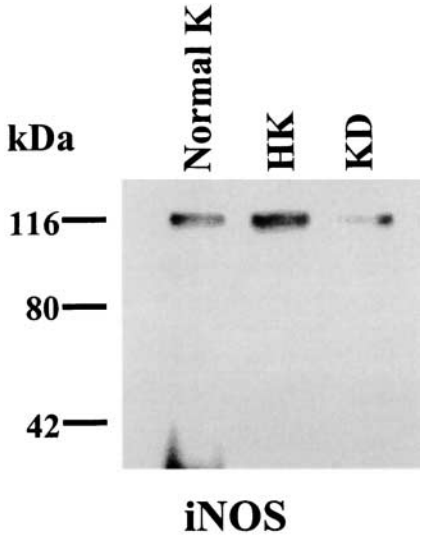

FIGURE 6. A Western blot showing the expressing iNOS in the renal outer medulla from rats on a high-K (HK) diet, a normal K diet (normal), and on a K-deficient (KD) diet.

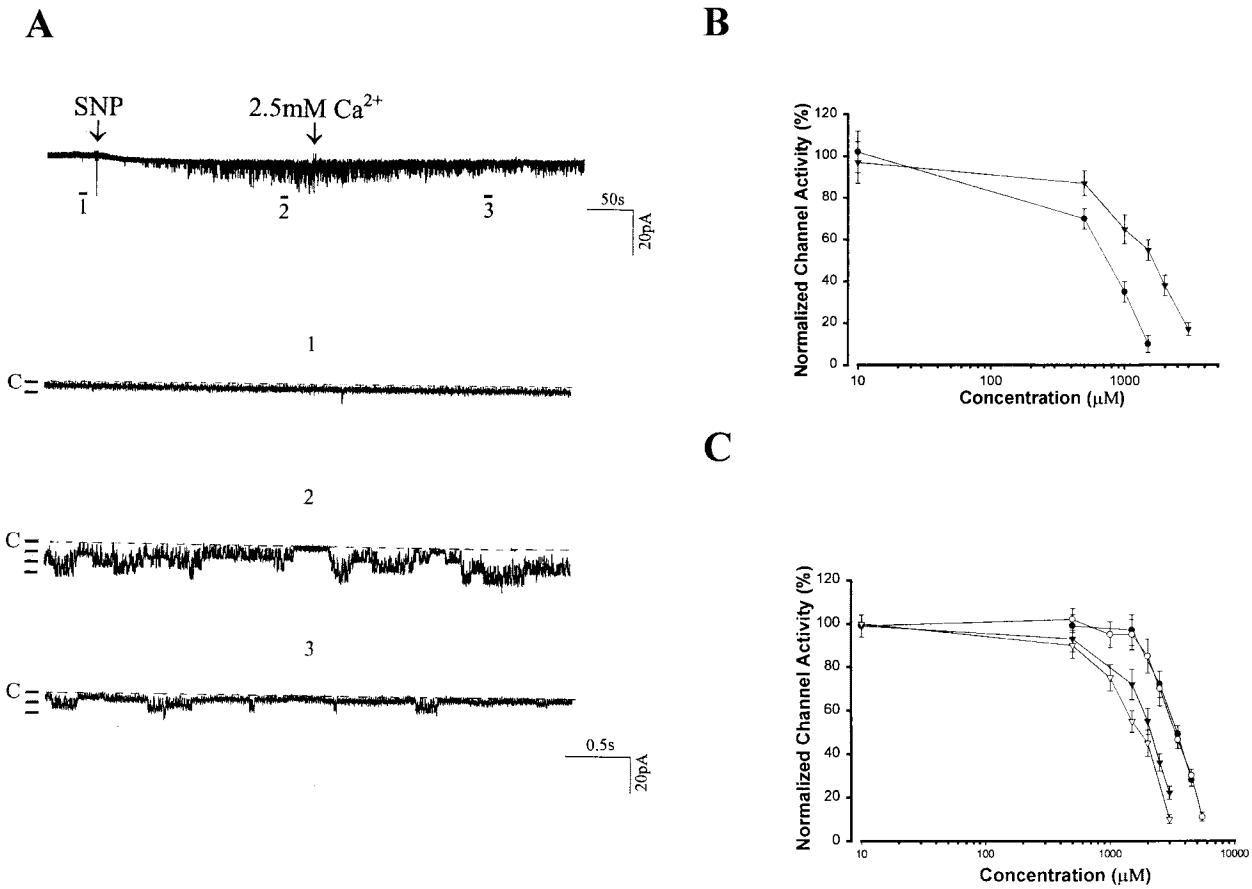

FIGURE 7. (A) A channel recording showing the effect of increasing the extracellular Ca^{2+} on the 70-pS K channel in the presence of 20 μM SNP in the mTAL from rats on a KD diet. The top trace is the time course of experiments, and the three parts indicated by numbers are extended to show the fast time resolution. (B) The effect of the external Ca^{2+} on channel activity in the mTAL from rats on a K-deficient diet in the presence 20 μM SNP (closed triangle) and in the absence of NO donor (closed circle). (C) The effect of the extracellular Ca^{2+} on channel activity in the mTAL from rats on a normal K diet in the presence (closed triangle) or in the absence of SNP (open triangle), and from rats on a high K diet in the presence (closed circle) or in the absence of SNP (open circle).

the dose–response curve of the extracellular Ca^{2+} effect shifted significantly to the right and K_i increased to ~1.8 mM. In contrast, addition of SNP has no significant effect on extracellular Ca^{2+}-induced inhibition of channel activity in the mTAL from rats on an HK diet, whereas it increases K_i slightly from 1.8 to 2.2 mM in the tubule from rats on a normal K diet (Fig. 7 C).

To further explore the role of NO in modulating the effect of the extracellular Ca^{2+} on the 70-pS K channel, we extended the investigation by examining the effect of raising the extracellular Ca^{2+} on channel activity in the presence of 200 mM L-NAME, an inhibitor of NOS, in the mTAL from rats on an HK diet. We confirmed the previous findings (Lu et al., 1998) that inhibiting NOS reduced channel activity (unpublished data). Moreover, inhibiting NOS increases the sensitivity of the 70-pS K channel to the extracellular Ca^{2+} because 2.5 mM Ca^{2+} decreased channel activity by 80 ± 6% (n = 10) in the presence of L-NAME (Fig. 8 A) in contrast to 30 ± 2% in the absence of L-NAME (Fig. 8 B). Moreover, Fig. 8 B shows that K_i falls from 3.4 mM in the absence of L-NAME to ~2 mM in the presence of L-NAME (n = 10). In contrast, L-NAME did not alter the sensitivity of the 70-pS K channel to raising the extracellular Ca^{2+} in the mTAL from rats on a KD diet, whereas the K_i decreased slightly from 1.8 to 1.3 mM in the mTAL from rats on a normal K diet (Fig. 8 C). The effect of L-NAME on the extracellular Ca^{2+}-induced inhibition was not the result of decreasing cGMP concentration since the effect of L-NAME was not mimicked by ODQ, an inhibitor of guanylate cyclase. Fig. 9 summarizes six experiments in which the effect of increasing the extracellular Ca^{2+} on channel activity was tested in the mTAL treated with ODQ from rats on an HK diet. It is apparent that ODQ did not significantly alter the sensitivity of the 70-pS K channel to the external Ca^{2+} because K_i is the same in the presence or in the absence of ODQ. The same observation that ODQ did not affect the response of the K channels to extracellular Ca^{2+} was

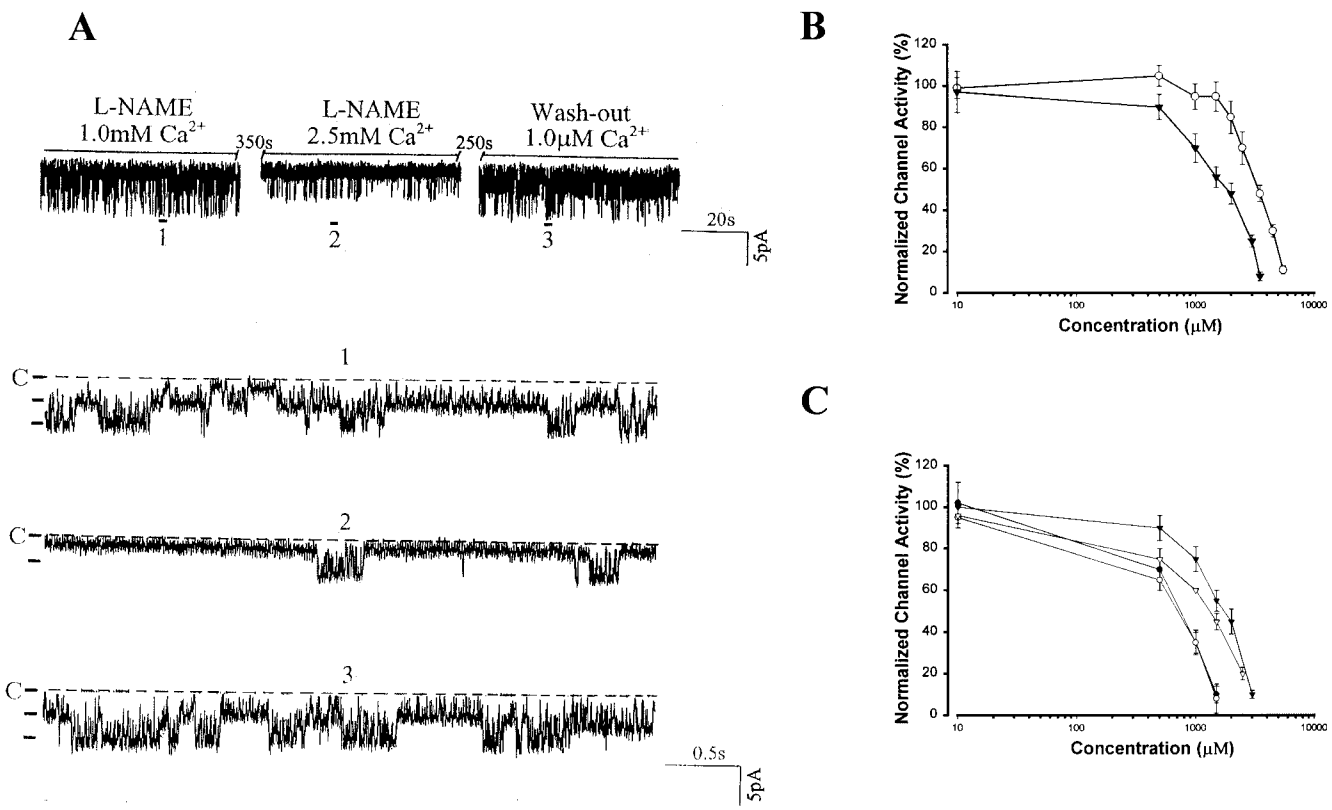

FIGURE 8. (A) A channel recording showing the effect of extracellular Ca^{2+} on the 70-pS K channel in the presence of 0.2 mM L-NAME in the mTAL from rats on a high K diet. The channel closed level is indicated by "C," and the holding potential was 0 mV. The three parts of the trace indicated by numbers are extended to demonstrate the fast resolution. (B) The effect of the external Ca^{2+} on the activity of the 70-pS K channel in the mTAL from rats on a high K diet in the presence of (closed triangle) and in the absence of 0.2 mM L-NAME (open triangle). (C) The effect of the extracellular Ca^{2+} on channel activity in the mTAL from rats on a normal K diet in the presence (open triangle) or in the absence of L-NAME (closed triangle), and from rats on a K-deficient diet in the presence (open circle) or in the absence of L-NAME (closed circle).

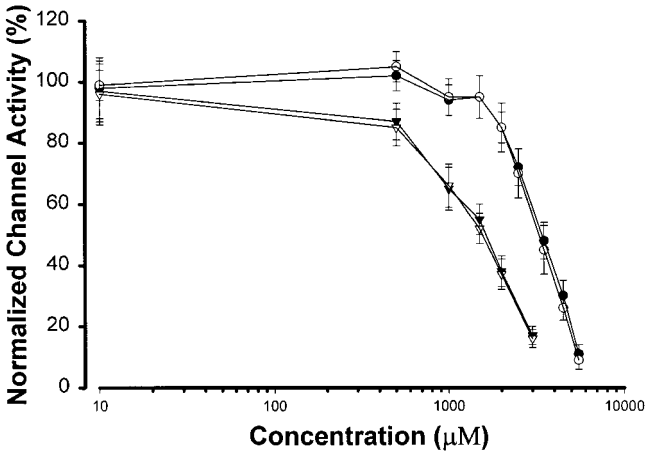

FIGURE 9. The effect of the external Ca^{2+} on the activity of the 70-pS K channel in the mTAL from rats on a high K diet in the presence (closed circle) and in the absence of ODQ (open circle). The triangles represent the experiment in which the effect of the extracellular Ca^{2+} on channel activity in the presence of 20 μM SNP was examined in the mTAL treated with ODQ (open triangle) or without ODQ (closed triangle) from rats on a K-deficient diet.

confirmed in the mTALs from rats on a normal K or on an HK diet (unpublished data).

The possibility that NO-induced decrease in the sensitivity of the apical K channel to the extracellular Ca^{2+} results from suppressing the cytochrome P450 metabolism of AA rather than from increasing cGMP was further explored in the mTAL treated with ODQ. Fig. 9 summarizes the results of five experiments in which the effect of SNP on the sensitivity of the apical K channels to the extracellular Ca^{2+} was determined in the tubules treated with ODQ from rats on a KD diet. Apparently, the treatment of the mTAL with ODQ did not affect the NO-induced modulation of the extracellular Ca^{2+} effect and K_i is still ~1.8 mM, which is the same as the value observed in the tubule treated with SNP in the absence of ODQ.

The notion that a decrease in NOS activity may be partially responsible for increasing the 20-HETE generation is also supported by the finding that the 20-HETE production was diminished significantly from 8.2 ± 1 to 5.8 ± 1 pg/μg protein (n = 5 rats) in the mTALs treated with 20 μM SNP from rats on a KD diet (Fig. 10). In contrast, treatment of the mTAL with 0.2 mM

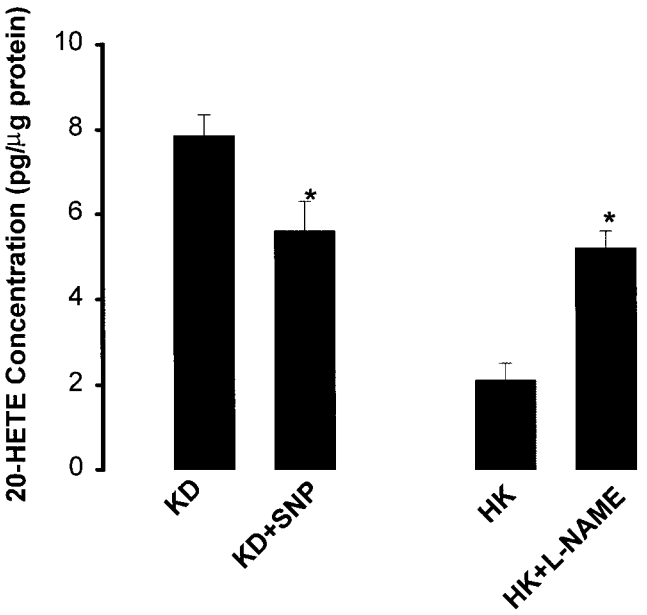

FIGURE 10. The 20-HETE concentrations in the isolated mTALs from rats on a K-deficient diet in the presence of 20 μM SNP and in the absence of SNP and from rats on a high-K diet in the presence of and in the absence of 0.2 mM L-NAME. The extracellular Ca^{2+} concentration was 1.8 mM.

L-NAME increased the 20-HETE production from 2.2 ± 0.3 to 4.1 ± 0.5 pg/μg protein (n = 4 rats) in the tubules harvested from rats on a high K diet. To further demonstrate that increasing NO concentration could attenuate the inhibitory effect of AA, we examined the effect of AA in the presence of NO. We confirmed the previous finding (Wang and Lu, 1995) that application of 5 μM AA inhibited the activity of the 70-pS K channel and decreased NP_o by 80 ± 6% (n = 5 patches). Moreover, application of 10 μM SNAP, a donor of NO, completely reversed the inhibitory effect of AA and increased the NP_o from 0.10 ± 0.02 to 1.1 ± 0.1 (n = 4; Fig. 11). The effect of SNAP is not the result of a direct stimulation of channel activity since addition of SNAP had no effect on channel activity in inside-out patches (unpublished data). Furthermore, the notion that the effect of SNAP on the AA-induced inhibition of channel activity results from suppressing the cytochrome P450 metabolism of AA was also confirmed by the finding that SNAP did not abolish the inhibitory effect of 20-HETE on channel activity in inside-out patches or cell-attached patches. Fig. 12 is a recording made in a cell-attached patch showing that application of 100 nM 20-HETE inhibited the 70-pS K channel by 90 ± 8% (n = 4 patches) in the presence of SNAP. Moreover, we confirmed the previous finding that the sensitivity of the 70-pS K channel to 20-HETE is the same in the mTAL from rats on a different K diet (Gu et al., 2001). Therefore, the results suggest that the effect of NO takes place before 20-HETE formation.

DISCUSSION

The apical 70-pS K channel has been shown to be a major type of the apical K channel responsible for K recycling in the rat mTAL (Wang et al., 1997b), and the reg-

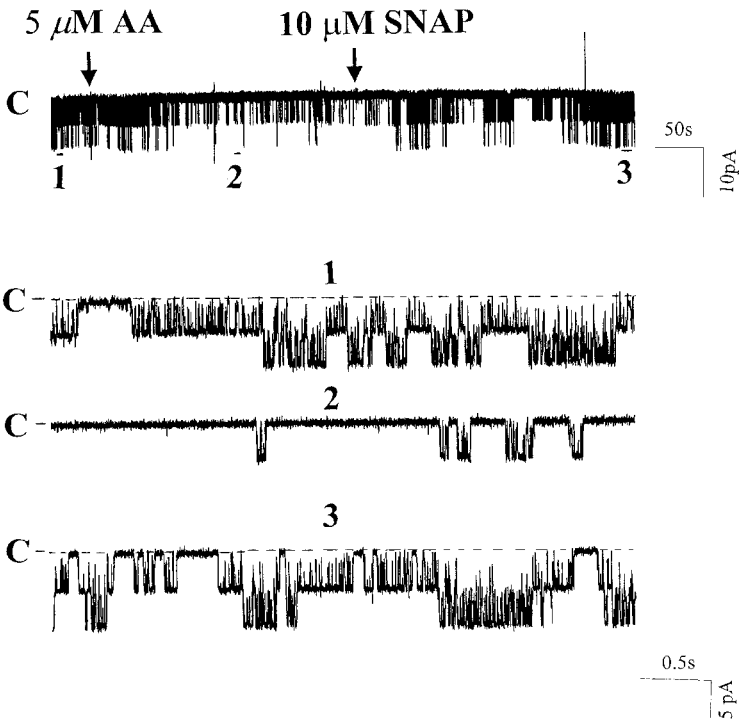

FIGURE 11. A channel recording showing the effect of 5 μM arachidonic acid on the activity of the 70-pS K channel in the presence of 10 μM SNAP. The experiment was performed in a cell-attached patch in the mTAL from a rat on a high K diet, and the pipette holding potential was 0 mV. The top trace shows the time course, and three parts of the trace are extended to demonstrate the fast time resolution.

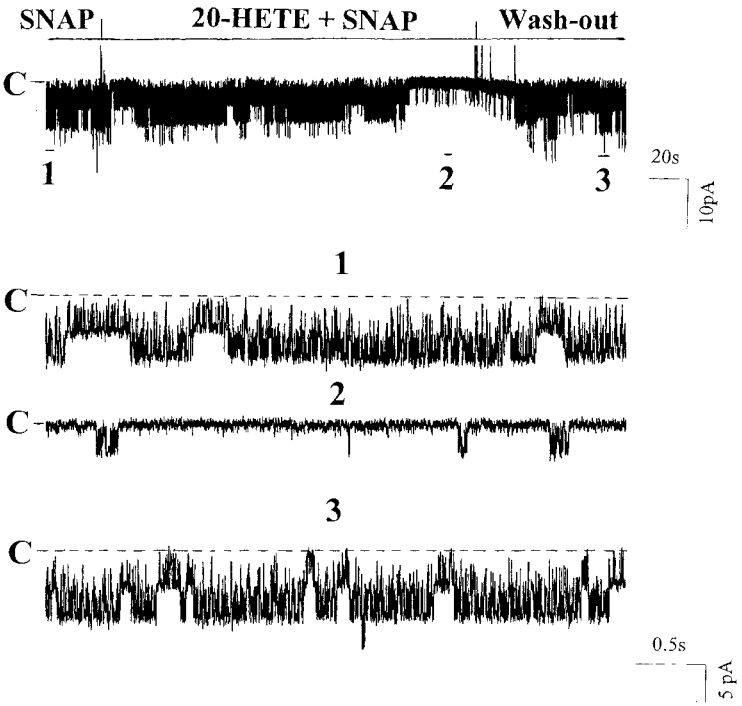

FIGURE 12. A channel recording demonstrating the effect of 100 nM 20-HETE on the activity of the 70-pS K channel in the presence of 10 μM SNAP. The experiment was performed in a cell-attached patch, and the holding potential was 0 mV. The top trace is the time course of the experiment, and three parts of the data are shown at a fast time resolution.

ulation of the K channel has been studied extensively (Wang and Lu, 1995; Liu et al., 1999, 2000). In the present study, we have demonstrated that the response of the 70-pS K channel to increasing the extracellular Ca^{2+} is enhanced in the mTAL from rats on a KD diet, whereas the response is diminished in the tubule harvested from the rats on an HK diet. The effect of the extracellular Ca^{2+} on the apical K channels does not result from a direct Ca^{2+}-mediated blockade because increasing the intracellular Ca^{2+} to the extent observed during raising extracellular Ca^{2+} had no significant effect on channel activity in inside-out patches (Wang et al., 1996). Our previous investigation suggested that the effect of the extracellular Ca^{2+} was most likely mediated by the CaR, which is expressed in the basolateral membrane of the mTAL (Hebert, 1996; Riccardi et al., 1996). This conclusion was based on the observation that application of CaR agonists (such as neomycin; Brown et al., 1993) mimicked the effect of the extracellular Ca^{2+} and inhibited the apical 70-pS K channel in cell-attached patches even in the Ca^{2+}-free bath solution. Moreover, neomycin had no direct effect on channel activity in inside-out patches (Wang et al., 1996).

The CaR has been found to be present in the proximal tubules, TALs, and collecting ducts (Hebert, 1996; Riccardi et al., 1996). The physiological role of CaR in the mTAL may include regulating the transepithelial Ca^{2+} reabsorption and transcellular Na transport (Hebert, 1996; Riccardi et al., 1998). The mTAL is an important site for renal reabsorption of the filtered Ca^{2+} load and Na. The Ca^{2+} reabsorption in the mTAL is driven by the lumen-positive potentials (Greger, 1985; Hebert, 1996). An increase in plasma Ca^{2+} concentrations is expected to stimulate the basolateral CaR in the mTAL and, accordingly, inhibit the apical K conductance. Since the apical K channels play an important role in K recycling that is involved in generating the positive transepithelial potential (Giebisch, 1998), inhibiting the apical K channels can diminish the lumen-positive potential and leads to inhibition of Ca^{2+} reabsorption in the mTAL. As the K recycling is also essential for maintaining the function of Na/K/Cl cotransporter, inhibiting the apical K channels by a high extracellular Ca^{2+} is expected to block the cotransporter and decrease Na reabsorption in the mTAL. This is important for reducing the risk of forming Ca^{2+} crystal because a large fluid volume allows increasing Ca^{2+} excretion without raising the luminal Ca^{2+} concentration significantly.

Although the signaling usage of the CaR in the mTAL is not completely understood, the finding that inhibiting soluble phospholipase A2 abolished the effect of increasing extracellular Ca^{2+} indicates that the effect is produced by an AA-dependent pathway (Wang et al., 1997a). Moreover, two lines of evidence suggest that the cytochrome P450 metabolite of AA (20-HETE) is an important mediator for the effect of stimulating CaR. First, inhibiting cytochrome P450 metabolism of AA blocked the effect of raising the extracellular Ca^{2+}. In contrast, inhibiting cyclooxygenase did not abolish the effect of increasing the extracellular Ca^{2+}. Second, stimulating CaR increased the 20-HETE production, which has been shown to block the apical 70-pS K chan-

nel (Wang and Lu, 1995). A large body of evidence has indicated that 20-HETE plays an important role in regulating Na transport in the mTAL. In addition to inhibiting the apical K channels, 20-HETE inhibits the Na/K/Cl cotransporter in the mTAL (Escalante et al., 1991). Therefore, the net effect of 20-HETE in the mTAL is to diminish the NaCl reabsorption. It has been demonstrated that 20-HETE production was significantly lower in response to a high salt intake in the renal tubule from Dahl salt-sensitive rats than those from Dahl salt-resistant rats (Ma et al., 1994). This may result in increasing NaCl reabsorption and high blood pressure in Dahl salt-sensitive rats.

Recently, a large body of evidence suggested that the 20-HETE production is regulated by nitric oxide (NO; Ahn et al., 1994; Mohaupt et al., 1994; Shin et al., 1999). It has been demonstrated that NO specifically inhibits P450 ω-hydroxylase, whereas it has no effect on epoxygenase activity (Oyekan et al., 1999). Three NOS isoforms, iNOS, nNOS, and eNOS, have been detected in the kidney (Morrissey et al., 1994; Bachmann et al., 1995; Kone and Baylis, 1997). Although their distribution in the renal tubules is still not completely understood, several studies have confirmed that iNOS and nNOS are present in the mTAL (Ahn et al., 1994; Mohaupt et al., 1994; Shin et al., 1999). The classical effect of NO is to stimulate guanylate cyclase, which in turn increases the cGMP production (Lincoln et al., 1996). We have demonstrated previously that NO stimulated the apical 70-pS K channel by a cGMP-dependent pathway because cGMP can mimic the effect of NO (Lu et al., 1998). However, three lines of evidence exclude the possibility that the NO-induced decrease in the sensitivity of the K channels to the extracellular Ca^{2+} is mediated by a cGMP-dependent pathway. First, addition of NO donor can still decrease the sensitivity of the K channels to the extracellular Ca^{2+} in the mTAL treated with ODQ. Second, inhibiting guanylate cyclase did not mimic the effect of L-NAME that enhanced the response of the K channels to the extracellular Ca^{2+} in the mTAL from rats on an HK diet. Third, application of NO donor reversed the inhibitory effect of AA but not 20-HETE. This suggests that the effect of NO results from decreasing 20-HETE concentration rather than increasing cGMP. Therefore, we speculated that the NO-induced decrease in the sensitivity of the K channels to the extracellular Ca^{2+} results from inhibiting cytochrome P450 metabolism of AA. This notion also is supported by the observation that the addition of NO donor decreases the 20-HETE concentration in the mTALs from rats on a KD diet, whereas inhibiting NOS increases the 20-HETE concentrations in the tubule from rats on an HK diet.

In the present study, we have observed that a high K intake significantly increases, whereas a low K intake decreases, iNOS expression in the mTAL. In addition, we recently have shown that a dietary K intake regulates the 20-HETE production in the mTAL: a low K intake increases, whereas a high K intake decreases the 20-HETE concentration (Gu et al., 2001). An increase in 20-HETE concentrations observed in the rats on a KD diet is closely correlated with a decrease in the 70-pS K channel activity (Gu et al., 2001). Moreover, either inhibiting cytochrome P450 ω-hydroxylation of AA or addition of NO donors increased the channel activity significantly in the mTAL from rats on a KD diet. This suggests that increasing the cytochrome P450 metabolism of AA and decreasing NO production may partially be responsible for decreasing the apical 70-pS K channel activity in the mTAL from rats on a KD diet. Also, it is possible that a decrease in NOS activity may, at least in part, be responsible for increasing 20-HETE production in the mTAL.

It has long been observed that epithelial transport function and urinary concentrating ability in the mTAL were compromised severely during hypokalemia (Rutecki et al., 1982; Senba et al., 1984; Unwin et al., 1994). Our present finding that the sensitivity of the apical K channel to the extracellular Ca^{2+} concentration is significantly enhanced in the mTAL from rats on a KD diet may be useful to explain why some pathophysiological observations occurred during hypokalemia. We speculate that the CaR is stimulated already even in the presence of physiological concentrations of extracellular Ca^{2+}. Indeed, this notion is supported by the observation that increasing extracellular Ca^{2+} to 0.5 mM significantly stimulated the 20-HETE formation in the mTAL from rats on a KD diet. In contrast, the same increase did not significantly affect the 20-HETE concentration in mTAL from rats on a normal K or on an HK diet. Also, the observation that decreasing extracellular Ca^{2+} increased the basal activity of the K channel in the mTAL from rats on a KD diet supports the possibility that K channel activity was suppressed by a physiological concentration of Ca^{2+}. Because K recycling is important for the function of the Na/K/Cl cotransporter, Na reabsorption is expected to be severely compromised in the mTAL from rats on a KD diet. However, since the ion transport in the mTAL could be sometimes in conflicting demand, it is unlikely that the signaling pathway described in this study would be the only mechanism by which K depletion impaired transport function in the mTAL.

Fig. 13 is a model of the mTAL cell illustrating the mechanism by which increasing extracellular Ca^{2+} inhibits the apical 70-pS K channel in rats on an HK diet or on a KD diet. We conclude that the response of the 70-pS K channel to the extracellular Ca^{2+} is upregulated in the mTAL from rats on a KD diet in comparison to the tubule from animals on a normal K diet or on an HK diet. Moreover, a decrease in NOS activity may be partially re-

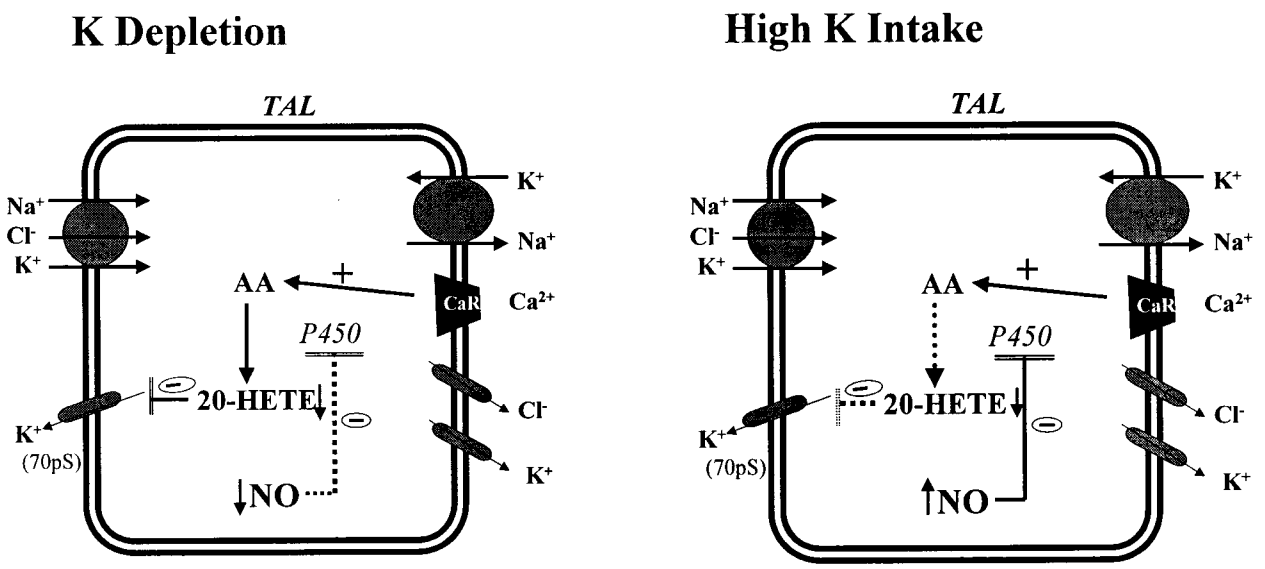

FIGURE 13. A model of the mTAL cell illustrates the mechanism by which external Ca^{2+} regulates the activity of the 70-pS K channels. The solid and dotted lines mean an enhanced and a diminished effect, respectively. The arrow and bar represent a stimulation and an inhibition, respectively.

sponsible for increasing 20-HETE production and for an augmented effect of the extracellular Ca^{2+} on the 70-pS K channel in the mTAL from rats on a KD diet.

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