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Research Article

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Transcriptional Activation of RACTK1 K^+ Channel Gene by Apical Alkalization in Renal Cortical Collecting Duct Cells

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Abstract

We have previously demonstrated that RACTK1 cDNA encodes a pH sensitive K^+ channel expressed in the apical side of renal collecting tubule cells. To determine whether extracellular pH induces the RACTK1 gene expression in the renal cortical collecting duct (CCD) cells, we measured mRNA of the RACTK1 using cultured rabbit CCD cells. Alkalization of incubation medium activated the transcription of the RACKTK1 gene in a time- and dose-dependent manner after 1 h, and reached a maximal level after 12 h. To examine whether the stimulation of mRNA by alkalization of body fluid occurs also in vivo, mRNA levels were measured in mice loaded with acid or alkali. The RACTK1 mRNA was increased in association with the rise in urinary pH. To examine side face of the effect of pH on stimulation of mRNA, we observed the effect of pH in the apical or the basolateral side in the preparation where CCD cells were cultured on filter membrane supports. Alkalization of the apical side but not of the basolateral side, was shown to be a determinant in inducting the RACTK1 mRNA. These findings suggest that, in addition to rapid direct regulation of RACTK1 K^+ channel conductance by intracellular pH, this channel is also regulated by the changes in luminal pH through synthesis of channel protein by transcriptional activation. (*J. Clin. Invest.* 1996. 98:474–481.) Key words: kidney • transcription • potassium channel • collecting tubule cell • alkalization

Introduction

Changes in the concentration of K^+ in hyperkalemia or hypokaremia can be life-threatening. The kidney plays a key role in regulating the extracellular K^+ content, since an amount equivalent to about 90% of ingested K^+ is excreted in the urine. Early micropuncture and microperfusion studies have firmly established that the initial and cortical collecting duct (CCD)[1] are the main sites for final fine regulation of urinary K^+ excretion (1–6). Two types of cells, principal cell and intercalated cell, are present in the CCD. The principal cell is responsible for K^+ secretion and Na^+ reabsorption (7, 8), whereas the intercalated cell is mainly involved in K^+ reabsorption and proton secretion via an apical K^+/H^+ pump (9–11). Various factors are known to control urinary secretion of potassium. Urinary secretion of potassium is increased by variety of factors including aldosterone, high potassium intake, and metabolic alkalosis. Increased intake of potassium is followed by prompt augmentation of urinary potassium excretion (12). Injection of aldosterone increases potassium excretion and decreases sodium excretion with a latency period of 1–2 h (13). Ultimately, the amount excreted is determined by the secretory activity of distal tubules and cortical collecting tubules. Majority of the studies on the cellular mechanisms of K^+ excretion have been achieved by the microperfused tubules, leading to the findings that the alteration of K^+ permeability in the luminal membrane of the collecting duct is important as a driving force for K^+ excretion. Lowering the luminal pH of isolated perfused rabbit collecting tubules depresses potassium excretion (14). Thus, the epithelial K^+ channel in the apical membrane of the collecting tubule is one of the most important determinant factors in the potassium excretion under hormonal regulation and acid-base metabolism. Recently, several types of cDNA encoding K^+ channels have been reported (15–17). Isolation of the candidate genes hitherto have substantiated more precisely the mechanism of the regulation in the mRNA level.

We have cloned cDNA encoding a voltage-independent K^+ channel from the rabbit renal cortical collecting duct (RACTK1) (18). The RACTK1 K^+ channel is characterized by inward-rectified I-V relation, and single channel conductance of 80 pS, which is blocked by Ba^{2+} but not by tetraethyl ammonium. The most striking feature is that the variation of cytoplasmic pH within physiological range dramatically influences the opening of the K^+ channel. An immunohistochemical analysis has shown that the RACTK1 is localized in the luminal membrane of the cortical collecting tubules (19). Therefore, the RACTK1 is one of the candidates to regulate urinary K^+ excretion. To assess the physiological significance of the RACTK1 in the kidney, we examined the regulation of the RACTK1 gene expression by the factors known to modify potassium excretion in vivo. We found that pH is important in the transcriptional regulation of K^+ channel in addition to the direct modulation of the function of K^+ channel.

Methods

Cell culture. SV40-transformed rabbit renal collecting duct (CCD) cells [20] were cultured in DMEM: HAMF12 medium, supplemented with 10% fetal calf serum (FCS), penicillin, streptomycin, and 25 mM Hepes (pH 7.4) (Sigma Chemical Co., St. Louis, MO). CCD cells were harvested on either 10-cm-diameter plates or microporous membrane (Falcon, Cell Culture Insert). Experiments were per-

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1. *Abbreviations used in this paper:* CCD, cortical collecting duct; DER gene, delayed early response gene; GAPDH, glyceraldehyde-3-3-phosphate dehydrogenase; IE gene, immediately early gene, NHE, Na+/H+ exchanger.

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formed on cells of three to six passages from primary transformants. To promote differentiation of CCD in vitro, we coated 10-cm cell culture dishes with 5mg/ml rat tail collagen. Cells (2×10^6) were evenly plated on the dishes and examined for morphological changes after 24 h. After the cells had grown to confluence on the collagen coated dishes, they were placed in quiescent FCS-free medium 48 h before the experiments. Then cultured CCD cells on 10-cm-diameter plates were incubated at various pH (7.0, 7.2, 7.4, 7.6, 7.8, 8.0) for various time intervals. The quiescent FCS-free DMEM: HAMF12 (1:1) conditioning media revealed pH 7.3–7.5 just before the experiments. Assuming that the standard pH of the medium is pH 7.4, we acidified or alkalized the medium by 0.4 pH unit by either adding HCl (pH 7.0) or NaOH (pH 7.8). This procedure did not affect the osmolality of the media.

In vivo acid or alkali loading to mouse. Male mice were allowed to access freely to animal chow and tap water and maintained for 1 wk, and then the tap water were changed to those containing either 0.01, 0.04, 0.1, 0.5 M NH_4Cl or 0.01, 0.04, 0.1, 0.5 M $NaHCO_3$ sterile water. After three days, urine pH and blood pH were measured and then the kidney of the mice were isolated for the sample of RNA.

Probe preparations. A rabbit or mouse RACTK1 cDNA fragment was amplified by the polymerase chain reaction as described (21). The sequences of the forward (5′ -CACCGATTCCTGTAGCACTTCC-3′) and reverse (5′- TGCCTGAATCACTGCTGC-3′) primers were used to amplify a 751-bp fragment. The polymerase chain reaction fragment was then subcloned and sequenced by the dideoxy chain termination method to confirm its authenticity.

Total RNA extraction and Northern blotting analyses. Total cellular RNA was extracted from cultured cells or kidney of mice by Isogen (Nippon Gene, Tokyo, Japan). Aliquots of total RNA, each consisting of 20 μg were fractionated on a 20% formaldehyde-agarose gel and transferred to nitrocellulose filters. The filters were hybridized at 68°C for 2 h with a random-primed, ^{32}P-labeled RACTK1 DNA probe in Quikhybri solution (Stratagene, La Jolla, CA). The hybridized filters were then washed in 300 mM sodium chloride, 3 mM sodium citrate, and 0.1% sodium dodecyl sulfate solution at 55°C and autoradiographed with Kodak XAR film at −80°C for 24–72 h. The filters were washed by boiling and rehybridized with a rabbit glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe to correct for differences in RNA loading (22). The filters were then scanned and radioactivity was measured on a Phosphor Imager running the Image Quant software (Molecular Dynamics, Sunnyvale, CA). The resultant densitometrical data of RACTK1 mRNA were compared to their GAPDH mRNA and expressed as the ratio. RNase protection assay was performed (23) with 20 μg RNA from CCD cells by using a kit (RPAII, Ambion).

Total protein extraction and Western blotting analyses. Whole-cell lysates were prepared from cultured CCD cells in a lysis buffer containing 1% SDS and 10 mM Tris, pH7.4. After incubation of the cells for 2 h at 4°C, they were centrifuged (1000 rpm, 1 min) and the supernatants were subjected for western blotting. Protein content in the CCD was determined according to the method of Lowry as modified for the DC protein assay (Bio-Rad Laboratories, Richmond, CA). 20 μg of total protein from CCD cells was fractionated by 10% SDS-PAGE, and the protein transferred to a polyvinylidene difluoride membrane (Immobilon-P membrane; Millipore Corp., Bedford, MA). The membrane was incubated with 5% skim milk in Tris-buffered saline for 1 h to block nonspecific absorption. RACTK1 was subsequently detected by incubating the membrane with a 1:2000 dilution of polyclonal anti–rabbit RACTK1 antibody for 1 h at room temperature followed by incubation with horseradish peroxidase-conjugated goat anti–rat IgG (1:20,000) for 1 h. Immune complexes were visualized using the ECL detection systems (DuPont NEN, Boston, MA). The immunoblot bands were measured by densitometric analysis of the autoradiograph.

Nuclear run-on assay. The cells ($\sim 10^7$) were incubated at different pH medium for 3 h and then scraped to make cell suspensions. Nuclear run-on assay was performed according to the methods reported elsewhere (24). Briefly, the cells were disrupted in the hypotonic solution (20 mM Tris, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM DTT, pH 7.5) followed by homogenization with 10 strokes in Dunce type homogenizer. The pellet was corrected by centrifugation (3000 rpm, 15 min, Swing rotor) and then suspended in the hypertonic solution (20 mM Tris, 0.2 mM EDTA, 5 mM $MgCl_2$, 1 mM DTT, 20% glycerol, pH 7.9).

Homogenization allowed the sample to release nuclei, which was confirmed by microscopic examination in Gimza staining. Repeating this procedure yielded the isolated nuclei to about 90% and then the nuclei were isolated by ultracentrifugation (40 K, 60 min). XhoI-digested plasmid DNA with or without (vehicle) RACTK1 cDNA (10 μg) were gel-purified and transferred to the nylon membrane (Hybond-N, Pharmacia, Piscataway, NJ). Labeling, hybridization and washing the target filters were performed under the described (23).

Statistical analysis. Quantitative data were described as mean±SE. Statistical analyses were performed using Student's *t* test or ANOVA as appropriate.

Results

Expression of RACTK1 mRNA in the CCD cells. At first, we cultured CCD cells under various conditions to find the best condition to detect RACTK1 mRNA. The confluent cells in collagen coated dish maintained at least 48 h in quiescent FCS-free media gave rise double 2.2 kb, 1.8 kb RACTK1 messages, suggesting that RACTK1 was highly expressed in this setting. Thus we employed this condition throughout the experiments. Addition of aldosterone or dibutylic cAMP and changes in K concentration did not alter RACTK1 mRNA levels when maintained for 24 h (Fig. 1). However, pH altered RACTK1 mRNA when we incubated the cells under these conditions for 3 h. The results lead us to speculate that the changes in pH at least in these cells may augment RACTK1 mRNA during earlier time periods. Since two bands were detected by the present probe, RNase protection assay was performed using the antisense single strand made by the same probe with RNA sample extracted in various pH at 3 h (Fig. 2). A single band was demonstrated at any pH, suggesting that the two bands may be derived from alternative processing during poly A tailing. To confirm this, we performed 3′RACE to amplify the tail, resulting in four amplified fragments, two of which were the alternative length of poly A tailing (data not shown).

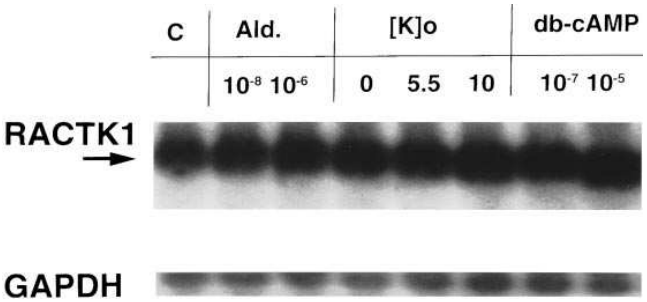

Figure 1. RACTK1 mRNA expression in CCD cells by exposure to aldosterone, K^+, db-cAMP for 24 h. The CCD cells were grown on collagen matrix in the quiescent condition. Then the cells were incubated for 24 h under control conditions (*C*), with aldosterone (*Ald.*), K^+ (*[K]$_o$*, mM/l), and dibutylic cAMP (*db-cAMP*) at the respectively given concentrations. Total RNAs were extracted from these cells, and subjected to Northern analysis as described in Methods. The 2.2-kb RACTK1 mRNA and rehybridized GAPDH are shown.

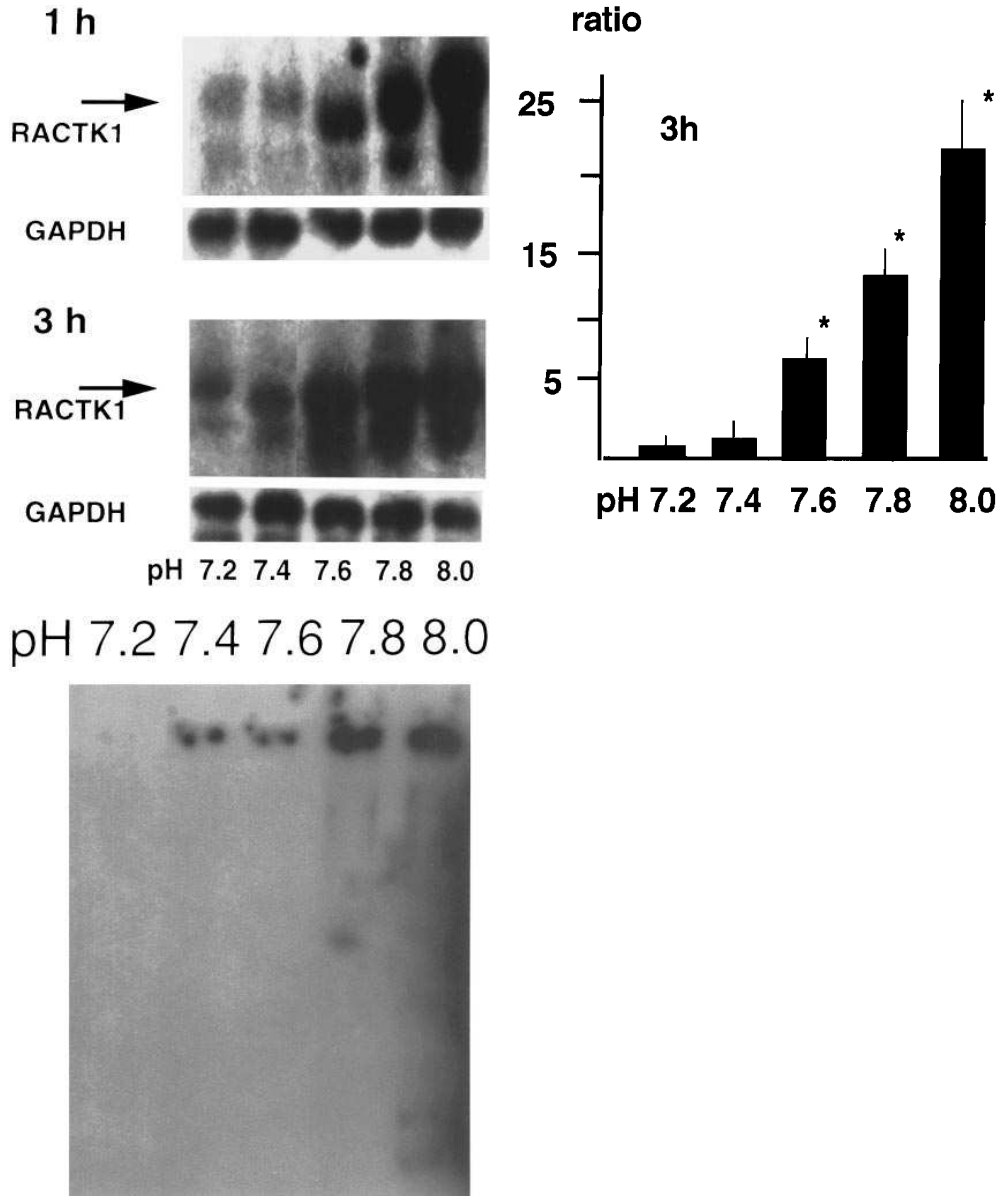

Figure 2. Influence of pH on the RACTK1 mRNA expression in CCD cells after 1 and 3 h. (*Left*) The CCD cells were grown on collagen matrix in the quiescent condition, then incubated for 1 or 3 h at the given pH. Total RNAs were extracted from these cells, and subjected to Northern analysis as described in Methods. The arrow-indicated 2.2-kb RACTK1 mRNA and rehybridized GAPDH are shown. Densitometrical ratios of the RACTK1/GAPDH are shown as a function of pH. (*Right*) The mean±SE of five experiments with 3-h incubations are presented as the fold-increases against control (pH 7.2). $*P <$ 0.01. (*Lower right*) RNase protection assay was performed with the RNA obtained from the cells incubated fro 3 h at the given pH. The probe sequence is the same as used in the Northern blot, and the antisense single strand was labeled for the probe.

Induction by various pH of RACTK1 mRNA in CCD cells. Influence of various pH on the RACTK1 mRNA of CCD cells are shown in Fig. 2. Although the level of RACTK1 mRNA from medium at pH 7.2 was low, the level increased in response to the elevation of pH after 1 h or 3 h. When the mRNA level at pH 7.2 was taken as the control, the levels were significantly ($n = 5$, $P < 0.01$) increased at pH higher than 7.6. Within this physiologic range of pH, the cells appeared vivid and thus we maintained the cells up to 24 h. Compared to the control pretreated condition, changes in pH to 7.6, 7.8, and 8.0 significantly increased RACTK1 mRNA after 1 h, followed by further increases in mRNA up to 24 h incubation ($P < 0.05$ pH 7.4 at 1 h, $P < 0.01$ pH 7.6 at 1 h, pH 7.4 and 7.6 at 3 h, Fig. 3). When the mRNA levels were compared in each incubation time, significant differences were usually obtained (at 1 h, $P < 0.05$, 3 h, $P < 0.05$, 6 h, $P < 0.01$, 12 h, $P <$ 0.01, and 24 h, $P < 0.01$). Therefore, RACTK1 mRNA responded in a time-dependent manner when the bath pH was higher than pH 7.4 at least in these cells.

Induction by various pH of RACTK1 mRNA in the kidney of mice. RACTK1 is similarly distributed in the mouse as well as in the rabbit, in which renal vasculatures and the renal collecting duct express the protein (data not shown). We detected the RACTK1 message in RNA prepared from mice, who ingested bicarbonate or ammonium to vary urinary pH. All of the blood pH of acid or alkali loading mouse, ranged for 7.35 to 7.5, were not significantly different from that of the control. The RACTK1 mRNA levels were plotted against urinary pH (Fig. 4). Although the level of RACTK1 mRNA from urine lower than pH 5.5 was high, elevating urinary pH from 6.0 to 8.0 correlated to the RACTK1 mRNA levels.

Induction by various pH of RACTK1 mRNA in the cells on microporous membrane. Since ingestion of acids or alkali undertaken in this study was sufficient to influence urinary pH but not blood pH, the correlation of urinary pH to RACTK1 mRNA suggested that the effect was exerted from the luminal side of the renal tubules. To examine this issue more specifically, CCD cells were grown on the microporous membrane to

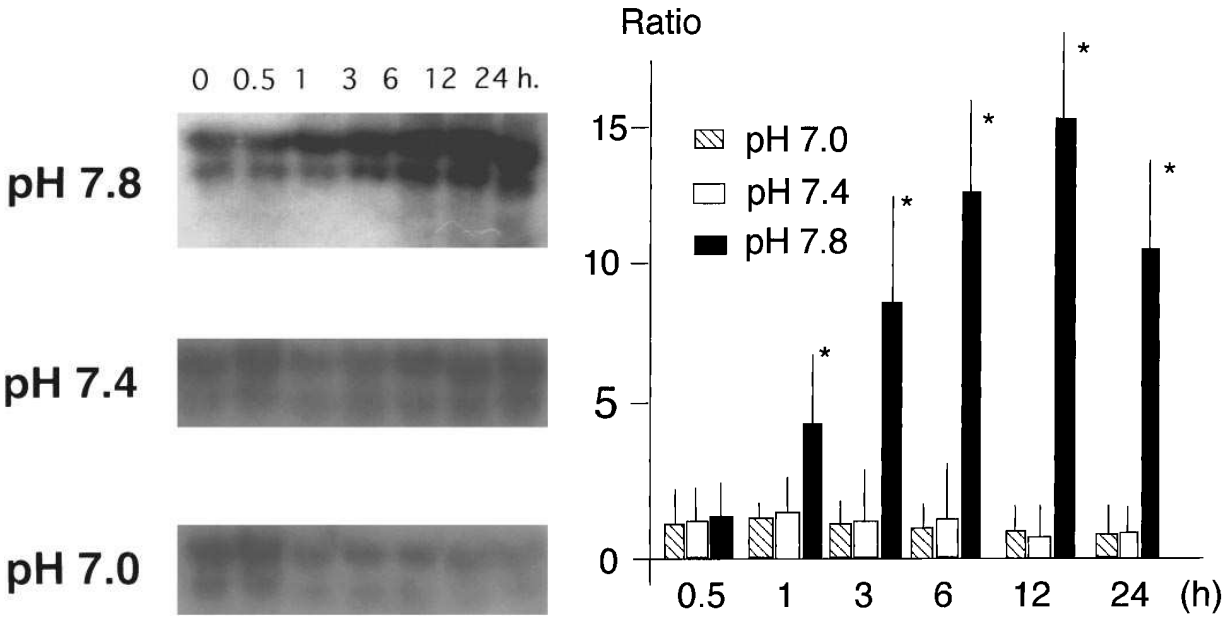

Figure 3. Influence of pH on the RACTK1 mRNA expression of CCD cells at various time-intervals. (*Left*) The CCD cells were grown on collagen matrix in the quiescent condition. Then the cells were incubated for indicated time at the given pH. Total RNAs were extracted from these cells, and subjected to Northern analysis as described in Methods. Densitometrical ratios of the RACTK1/GAPDH are shown as a function of incubation time. (*Right*) The mean±SE of five experiments at the given pH are presented as the fold-increases against control (the levels before treatments). $*P < 0.01$ compared to the control level.

make the epithelial polarity: The apical side of the cells represented the luminal membrane. Difference of the pH between the apical and the basolateral side was maintained during the experiments for 3 h, suggesting that the cell layers are tight enough to prevent leakage. When incubated for 6 h, the difference of pH was not maintained. The effects of changes in pH in the apical or the basolateral side on the RACTK1 mRNA were examined at 3 h (Fig. 5). The change of pH in the apical side but not in the basolateral side influenced the RACTK1 mRNA levels.

Effects of pH on RACTK1 protein levels in both CCD and mice renal medulla. We performed western blot analysis to determine whether the rise in RACTK1 protein is followed by the rise in mRNA induced by pH change. The antibody to RACTK1 recognized a major glycosylated band and a minor band with molecular masses of 31 kD (19). Fig. 6 shows the time course of pH-induced RACTK1 protein expression of 31 kD. Although the low pH (7.0) medium did not influence the density of the 31 kD band (data not shown), high pH medium (pH 7.8) increased the density after 6 h, and further increased up to 24 h of incubation. Therefore, the results of western blot suggested that the increased protein levels of the RACTK1 by alkalization is timely followed by the rise in mRNA.

Stability of RACTK1 mRNA. To determine whether alkali

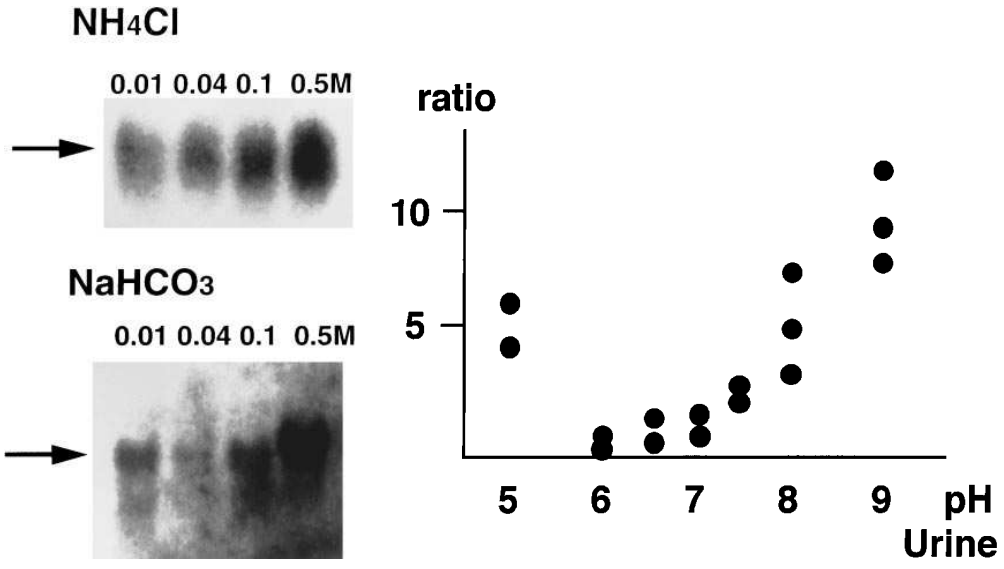

Figure 4. Effect of in vivo acid-base balance on the RACTK1 mRNA expression in the mouse kidney. (*Left*) Mice were maintained for 3 d with tap water containing the given concentration of NH_4Cl or $NaHCO_3$. The kidney were isolated for the RNA sample after the measurements of urinary pH, and subjected to Northern analysis as described in Methods. The arrows-indicate 2.2-kb RACTK1 mRNA. Densitometrical ratio of the RACTK1/GAPDH was measured. (*Right*) Each closed circle represents the ratio to untreated control mouse plotted against urinary pH.

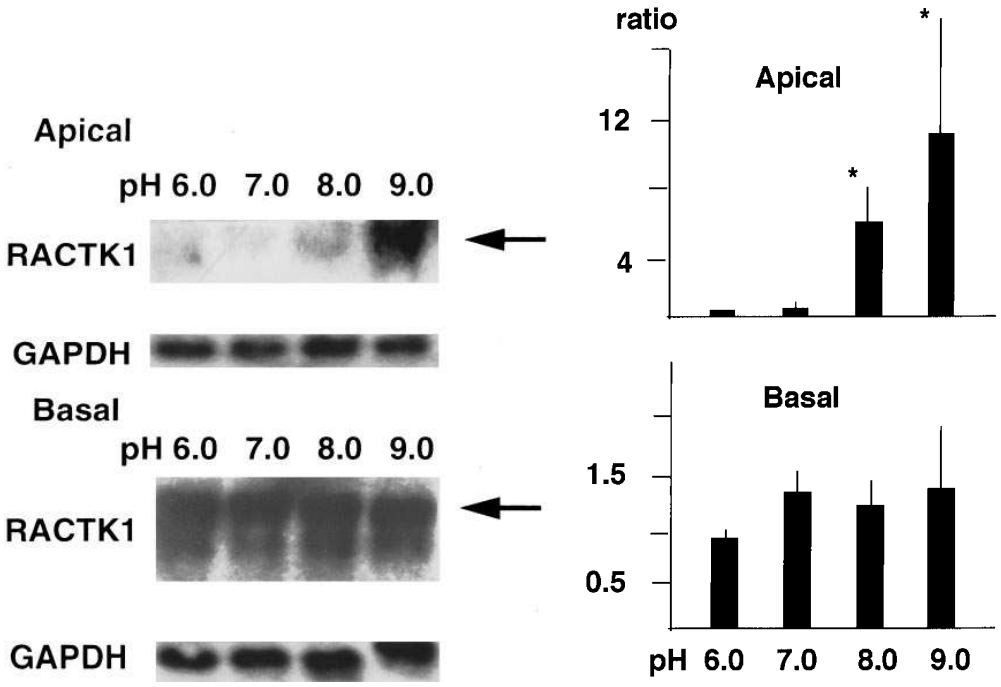

Figure 5. Effect of changes in pH in the apical or the basolateral side on the RACTK1 mRNA expression. (*Left*) The CCD cells were grown on microporous membrane supports to reach confluence. After 48 h of incubation with quiescent media, the cells were incubated for 3 h with the given pH of the apical or the basolateral side. Total RNAs were extracted from these cells, and subjected to Northern analysis as described in Methods. Arrows indicate 2.2-kb RACTK1 mRNA and rehybridized GAPDH. Densitometrical ratio of the RACTK1/GAPDH was measured. (*Right*) The mean±SE of five results in exchange of media with the apical (*Apical*) or the basal (*Basal*) side of the membrane supports are presented against control (the levels before treatments). $*P <$ 0.01.

affected the steady-state level of RACTK1 mRNA by decreasing its rate of degradation, we measured the RACTK1 mRNA half-life by using actinomycin D or cyclohexamide/anisomycin to block transcription and translation after stimulation of mRNA by alkalization. The RACTK1 mRNA or RACTK1/GAPDH ratio was decayed after the treatment of actinomycin D (Fig. 7). Although the mRNA level was high in pH 7.8, the half-life was about 2 h and was not changed by variations of pH (7.4 and 7.8), suggesting that a rise in pH activated the transcription rather than the degradation of the RACTK1 mRNA. The half-life of GAPDH was $\sim$ 3.5 h and unchanged by the alteration of pH. Moreover, both transcription and translation were blocked with the mixture of cycloheximide/anisomycin. Since the translation was blocked, the half-life of RACTK1 mRNA degradation was prolonged but not influenced by the pretreatment of the cell with pH (7.4 vs. 7.8).

Nuclear run-on assay was performed to reveal directly whether the change in pH influences the transcription of the RACTK1 mRNA. The labeled nucleus incubated at pH 7.8 for 3 h hybridized more than that at pH 7.4 to the RACTK1 containing plasmids (Fig. 8).

Discussion

Two types of cells, the principal cell and the intercalated cell are present in the CCD. The principal cell is responsible for K^+ secretion and Na^+ reabsorption (7, 8), whereas the intercalated cell is mainly involved in K^+ reabsorption and proton secretion via apical K^+/H^+ pump (9–11). In the present study, we found that the RACTK1 gene expression is regulated by pH in cultured SV-40–transformed rabbit CCD cells. These cells have some particular characteristics of principal cells (20).

Recently, various types of K^+ channel cDNA were isolated, and the regulation of their mRNA have been reported. There are two types of K^+ channels present in CCD cells, Ca^{2+}-activated large-conductance (maxi-K) and ATP-inhibitable small conductance types (K_{ATP}). The maxi-K^+ channels have been identified in the paical membranes of both thick ascending limb of Henle (TAL) (25) and CCD (26–28), but it is generally thought that this apical maxi-K^+ channel is not directly involved in K^+ secretion by this nephron segment. The K_{ATP} is believed to be involved in the regulation of K^+ secretion (11, 12). RACTK1 represents ATP inhabitable but CA^{2+}-activated intermediate K^+ channel (IK_{Ca}) (29), while ROMK1 (rat kidney outer medulla K^+ channel) (15) represents ATP inhibitable small conductance K^+ channel. Although the previous data suggest the importance of the small conductance K^+ channel in K^+ secretion, they are mainly based on the observations in the rat, in which CCD possesses maxi-K and the K_{ATP}. On the other hand the other data including ours have shown that CCD of the rabbit possesses the Ca^{2+}-activated K^+ channel which has conductance of $\sim$ 80 pS lower than maxi K^+. The role of the IKCa in K^+ secretion of the rabbit has not been established. Additionally, there are little data indicating whether aldosterone, pH and potassium intakes for example

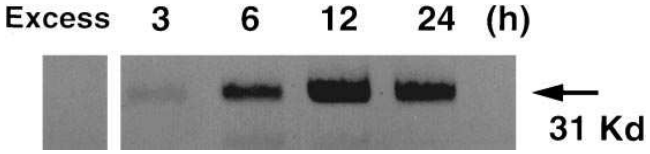

Figure 6. Western blotting analyses showing effect of pH on expression of the RACTK1 protein. The CCD cells were grown on collagen matrix in the quiescent condition. The cells were incubated for 3. 6, 12, 24 h at pH 7.8. Total proteins were extracted from these cells, and subjected to Western blotting analyses as described in Methods. Each lane contains 20 μg of the protein. Excess amount (10 μg) of peptide antigen was incubated with the primary antibody. The RACTK1 protein detected as 31 kd band indicated as arrow.

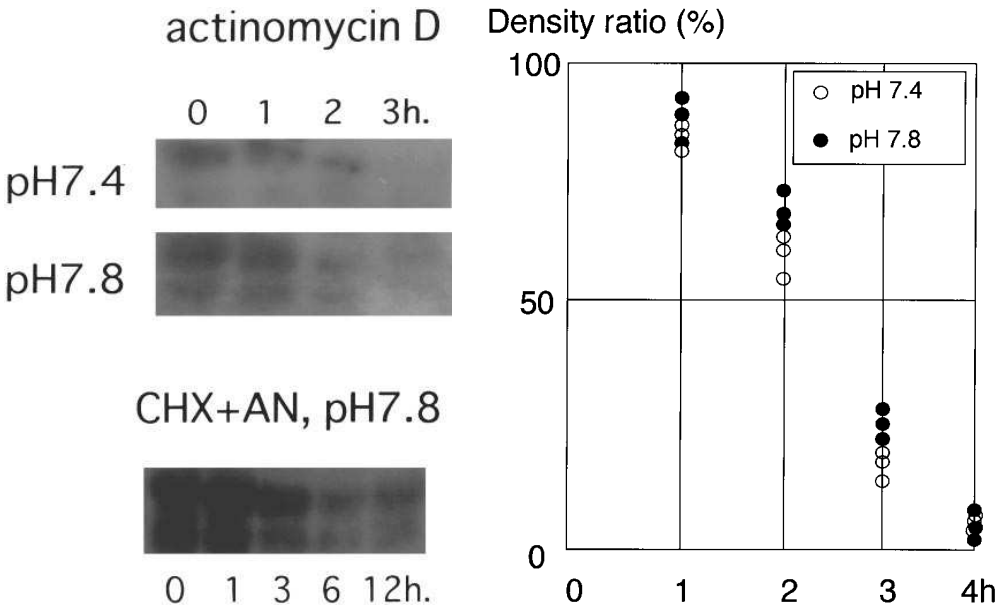

Figure 7. Effect of inhibitors on RACTK1 mRNA expression. The CCD cells were grown on collagen matrix in the quiescent condition. (*Left*) After the preincubation in quiescent media, CCD cells were incubated at pH 7.4 or at pH 7.8 for 12 h (time 0). These cells were treated with actinomycin D (10μg/ml; *upper panel*) or with cycloheximide (10 μg/ml: *lower panel*) and anisomycin (5 μM) at the given time periods. Total RNAs were extracted from these cells, and subjected to Northern analysis as described in Methods. (*Right*) Densitometrical ratio of the RACTK1/GAPDH was measured. The resultant ratio (100% at time 0) at pH 7.4 (*open circle*) or at pH 7.8 (*closed circles*) were plotted against the time after addition of actinomycin D.

dependently regulate K^+ excretion through the same K^+ channel. The small conductance K^+ channel may be important in the response to potassium intakes and/or aldosterone. The alteration in pH may regulate other channels responsible for K^+ secretion. It is of interest to note that the expression of ROMK1 may be also regulated by pH.

We examined several factors which are believed to influence urinary K^+ excretion in vitro; alteration of pH alone affected the expression of the RACTK1 mRNA. However, since we did not examine the physiological response to aldosterone or to fluctuation of K^+ concentration in the present CCD cells, we can not exclude the possibility that those factors other than pH could regulate the RACTK1 mRNA in vivo.

It has been reported that various factors regulate K^+ channel genes. For example, phosphorylation by protein kinase A and depolarization by KCl regulate the expression of voltage-dependent K^+ channel mRNA (22). Dexamethasone rapidly induces K^+ channel gene in pituitary cells (30). Acid-base balance influences K^+ excretion in the kidney, with alkalosis enhancing and acidosis inhibiting urinary K^+ excretion. The apical small conductance K^+ channel is exquisitely pH sensitive; a reduction of the bath pH from 7.4 to 6.9 completely blocks the channel activity in inside-out patches (11). However, the decrease of K^+ secretion in acidosis is not great as might be expected from the pH-induced decline of the apical K^+ conductance. Intracellular H^+ inhibits ROMK1 expressed in *Xenopus* oocytes (31). Although the increase in pH functionally activates several types of K^+ channels (18, 32), this is the first paper which explicitly shows the transcriptional activation of K^+ channel by extracellular alkalization.

On the other hand, pH alters the expression of many other molecules such as the accessory gene regulator (*agr*) in staphylococcus aureus (33) and cytochrome oxidase (34). Among ion transporting elements, Na^+/H^+ exchanger (NHE) is known to be regulated by external pH. Northern hybridization indicates that NHE-1 mRNA increases, whereas NHE-2 mRNA decreases when incubated with acidic media (35). H^+/K^+ ATPase can be also regulated by external pH and K^+ concentration; metabolic acidosis increases and alkalosis suppresses H^+/K^+ ATPase activity n the rat renal outer medulla. This stimulatory effect of metabolic acidosis appears to be additive to the effect of K^+ depletion, and possibly due to the regulation of the mRNA expression (36). Therefore, the transcriptional regulation of ion transporting elements by external pH may well be operated also in the kidney.

The present results provide two unique observations. The response of the RACTK1 mRNA to high pH was relatively rapid because only 1 h incubation provided a detectable change of the RACTK1 mRNA. So-called "immediately early gene (IE gene)" including *c-fos* and *egr-1*, plays some roles in cell proliferation and renal growth after ischemia (37). IE genes are activated within minutes, even in the presence of an inhibitor of protein synthesis (38–41). Among the proteins they encode are transcription factors, cytokines, membrane proteins, and cytoskeletal proteins (42–44). After a few hours, but still before the onset of DNA synthesis, the delayed early response genes (DER gene) are activated. In contrast to IE genes, their activation requires protein synthesis (43). They include genes that encode biosynthetic enzymes, secreted proteases, potential cytokines, transcription factors, cyclin family,

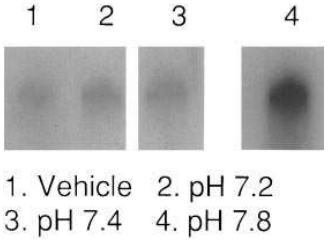

Figure 8. Nuclear run-on of RACTK1 mRNA. Nuclei were isolated from the cells incubated with different pH medium for 3 h. XhoI-digested plasmid DNA with or without (vehicle) RACTK1 cDNA (10 μg) were gel-purified and transferred to the nylon membrane (Hybond-N, Pharmacia). Labeling, hybridization and washing the target filters were performed as described (23).

nonhistone chromosomal proteins, and the water channel CHIP28 (45). The voltage-dependent L-type Ca^{2+} channel is also regulated by IE gene and may be involved in DER gene (46). The time course of the increase in the RACTK1 mRNA by high pH which was blocked protein synthesis inhibitors, is in timely accord with the DER gene. Although induction of IE genes are stimulated by an addition of serum to the cultured cells, RACTK1 mRNA was decreased by this treatment (data, not shown).

Alkalization of the luminal side but not basolateral side influenced the RACTK1 mRNA. Therefore, the luminal membrane of the collecting duct might possess a unique mechanism to induce the RACTK1 mRNA. The transcriptional activation of the RACTK1 gene by external pH might require some unknown mechanisms which are rapidly responsive and specific for the collecting tubules. Further studies are necessary to elucidate the mechanisms.

The fact that alkalization activated the transcription of the K^+ channel expression is quite compatible with the regulation of K^+ excretion in vivo in that alkalosis induces kaliuresis in mammals including human. Therefore, RACTK1 K^+ channel plays a role in K^+ excretion in response to acid-base metabolism. We may conclude that the transcriptional activation of RACTK1 K^+ channel gene is responsive to luminal alkalization, playing a role in K^+ excretion associated with acid-base metabolism.

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