TRPV4 as a flow sensor in flow-dependent K⁺ secretion from the cortical collecting duct

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THE TRANSIENT RECEPTOR POTENTIAL vanilloid-4 (TRPV4) was found to encode a Ca²⁺-permeable cation channel (7). The TRPV4 channel expressed in cultured cells or Xenopus laevis oocytes is activated by hyposmolality (7), shear stress (5), or moderate heat (8). Recent investigations have disclosed, by an in vitro microperfusion technique showing that net K⁺ secretion in the rabbit CNTs consisted of flow-dependent and flow-independent components and that CTX blocked the flow-dependent component (27). The requirement for the Ca²⁺ influx, however, has been controversial in flow-dependent K⁺ secretion via maxi-K⁺ channels, because maxi-K⁺ channels are stretch activated Ca²⁺-independent in rat CCDs (28). Therefore, it was reported that an in vivo kaliuretic response to volume expansion was lost in knockout mice through gene disruption of the maxi-K⁺ channel β1-subunit, which enhances the Ca²⁺ sensitivity of this channel (14). This result suggests that the essential role of a Ca²⁺ mobilization modulator, such as the stretch-activated cation channel, is to sense luminal flow. Although the mechanism has been described in detail for flow-dependent K⁺ secretion, no mechanosensitive molecular information has been available until now.

Abundant expression of TRPV4 channels has been detected in the kidney (7), but details regarding their localization are not available. Tian et al. (22) detected basolateral expression of TRPV4 using a sensitive antibody in all nephron segments under the ascending thin limb, except the macula densa, in rat and mouse kidneys. However, protein kinase C and casein kinase substrate in neurons (PACSIN3) were recently reported to be binding proteins of TRPV4 and to colocalize in the connecting tubule (CNT), and cortical collecting duct (CCD) (8). Taniguchi and Imai (20) found, using patch-clamp and in vitro microperfusion techniques, that the Ca²⁺-activated maxi-K⁺ channel in the luminal membrane of rabbit CNTs was apparently stretch activated in the presence of extracellular Ca²⁺; in addition, they demonstrated that the increase in luminal flow increased the K⁺ conductance in the luminal membrane, which was completely inhibited by charybdotoxin (CTX), a selective blocker of maxi-K⁺ channels (10, 18, 20).

Simultaneous stretch activation of maxi-K⁺ channels was reported in the apical membrane of A3 cells derived from a rabbit medullary thick ascending limb (19). In addition, the parathyroid hormone (PTH)-dependent luminal Ca²⁺ influx was flow dependently increased with stretch-activated Ca²⁺-permeable cation channels in the luminal membrane of rabbit CNTs (21). It is possible that K⁺ ions were secreted via maxi-K⁺ channels that were activated by luminal Ca²⁺ influx via stretch-activated Ca²⁺-permeable cation channels, because the maxi-K⁺ channels themselves are not stretch activated in rabbit CNTs (20) and A3 cells (19). This hypothesis was supported by observations using an in vitro microperfusion technique showing that net K⁺ secretion in the rabbit CCDs consisted of flow-dependent and flow-independent components and that CTX blocked the flow-dependent component (27). The requirement for the Ca²⁺ influx, however, has been controversial in flow-dependent K⁺ secretion via maxi-K⁺ channels, because maxi-K⁺ channels are stretch activated Ca²⁺-independent in rat CCDs (13). Therefore, it was reported that an in vivo kaliuretic response to volume expansion was lost in knockout mice through gene disruption of the maxi-K⁺ channel β1-subunit, which enhances the Ca²⁺ sensitivity of this channel (14). This result suggests that the essential role of a Ca²⁺ mobilization modulator, such as the stretch-activated cation channel, is to sense luminal flow. Although the mechanism has been described in detail for flow-dependent K⁺ secretion, no mechanosensitive molecular information has been available until now.

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luminal membrane of renal tubules (3). Thus the precise localization of TRPV4 is controversial and may depend on the antibody used.

In this study, we compared net K⁺ flux in CCDs isolated from either TRPV4−/− or TRPV4+/− mice with an in vitro microperfusion technique to explore the role of TRPV4 channels as a flow sensor in the mechanism underlying flow-dependent K⁺ secretion. We also investigated urinary K⁺ excretion in vivo in both types of mice, in which urine production had been accelerated by furosemide.

**METHODS**

**Mice.** TRPV4−/− mice with homozygous disruption of the TRPV4 gene (11) were backcrossed with TRPV4+/− C57BLq6 mice obtained from Clea Japan (Tokyo, Japan). TRPV4−/− mice of the sixth through ninth generations were used in this study. Both TRPV4−/− and TRPV4+/− mice were allowed free access to tap water and normal chow. We used both types of male mice (3–6 mo old) in all the experiments, which were approved by the Animal Experimental Committee of Jichi Medical University.

**In vitro microperfusion.** In preliminary experiments, we could not detect the net fluxes of Na⁺ and K⁺ in CCDs isolated from normal TRPV4+/− and TRPV4−/− mice with an in vitro microperfusion technique. Similar results were reported in rat CCDs, but the net fluxes of Na⁺ and K⁺ were augmented in CCDs isolated from rats pretreated with mineral corticoids (24). Thus we pretreated both male TRPV4+/− and TRPV4−/− mice with 2 mg/mouse 1-day−1 deoxy- corticosterone acetate (DOCA; Sigma, St. Louis, MO) subcutaneously (sc) for 5 days for accurate measurements of K⁺ and Na⁺ fluxes in mouse CCDs with the in vitro microperfusion technique.

**Either a TRPV4+/− or a TRPV4−/− mouse was anesthetized intraperitoneally (ip) with 40 mg/kg body wt (BW) pentobarbital sodium (Nembutal, Abbott, IL, or Somnopentyl, Sankyo, Tokyo, Japan), and its kidney was excised. We isolated CCDs from thin slices of renal cortex in chilled modified Collins’ solution (4°C) that contained (in mM) 14 KH₂PO₄, 14 K₂HPO₄, 15 KCl, 9 NaHCO₃, and 160 sucrose (pH 7.4). The CCDs were transferred to a bathing chamber mounted on an inverted microscope (IMT-2; Olympus, Tokyo, Japan). Single isolated CCDs were then perfused according to the technique reported by Burg et al. (2) with slight modifications (25). The net fluxes of water (Jₒ), K⁺ (Jₒ-K⁺), and Na⁺ (Jₒ-Na⁺) were measured at room temperature, when the tubular lumen was perfused at a perfusion pressure of 0.7 kPa applied to the inner perfusion pipette for the slower luminal flow (2–3 nl/min) or 1.2 kPa for the faster luminal flow (8–9 nl/min).

We obtained the net water flux across the perfused CCDs by measuring the inulin concentration in the perfusate and tubular effluent with the following equation

$$J_w = \frac{V_e}{L} \left( \frac{[\text{inulin}]_w}{[\text{inulin}]_L} - 1 \right)$$

where $V_e$ was the collection rate of effluent to an constant in volume pipette (in nl/min); $L$ was the length of the tube (in mm); and [inulin]ₜ and [inulin]ᵢ were the concentrations of inulin in perfusate and effluent, respectively. The ion flux (Jₒ) was obtained from the following equation

$$Jₒ = \frac{X[L(Jₒ + Jₒ) - \left(X\right)_{V_e}]}{[X]_{V_e} = \frac{[X]_J_o + \left([X]_i - [X]_o\right)}{L}}$$

where $[X]_i$ and $[X]_o$ were the concentrations of solute X in the perfusate and effluent, respectively. Samples of effluent were transferred into mineral oil equilibrated with water. The Na⁺ concentrations of samples were measured with sodium green (Molecular Probes, Eugene, OR) by using a continuous flow-through microfluorometer (NANOFLUO; WPI, Saratoga, FL) (29). For the measurement of inulin concentration, fluorescein isothiocyanate-inulin (FITC-inulin) (Sigma) was added at 10–100 μM into the perfusate as a marker of the inulin concentration. We obtained the inulin concentration from the fluorescense of FITC-inulin measured with the same microfluorometer (NANOFLUO, WPI). The K⁺ concentration was measured with the Ultramicroflamephotometer (AF-A707D; Apel, Tokyo, Japan) (25). The luminal flow rate was estimated from $V_e$, because $J_w$ was not significantly different from zero in any experimental conditions used in this study.

**Measurement of urinary K⁺ excretion in vivo.** Both types of male mice were anesthetized ip with 40 mg/kg BW pentobarbital sodium, and they were laid on a warmed bed (38–40°C). We opened the lower abdomen to insert glass tubing into the urinary bladder. To increase urine production, we continuously infused 2% NaCl solution from the tail vein at a rate of 20 ml b⁻¹ kg⁻¹ BW⁻¹ throughout the experiment. After the infusion of this solution for 1 h, urine was collected from the glass tubing into a polyethylene tube with a vacuum pump for 30 min as control urine. We then collected another urine sample for 10 min after urine production was further accelerated by iv application of 2 mg/kg BW furosemide. This urine collection was begun at 5 min after the application of furosemide. We also collected urine samples from both types of mice pretreated with DOCA, as described above, using the same protocol.

We measured the urine volume with a micropipette. The entire urine sample was carefully sucked into the plastic tip of a micropipette. It was then pushed forward by turning the volumetric screw until the urine reached the tip. A reading of the scale showed the urine volume. The urinary K⁺ concentration was measured with the flamephotometer IL 943 (Instrumentation Laboratory, Milan, Italy), and the urinary excretion of K⁺ was obtained from the urine volume and K⁺ concentration.

**Reagents.** CTX (Peptide Institute, Osaka, Japan) was dissolved in purified water at 100 μM, and the solution was stocked at −20°C. The stock solution of CTX was diluted at 1 μM in the luminal perfusate. Amiloride (Sigma) was also dissolved at 10 μM in the luminal perfusate. DOCA (Sigma) was suspended at 20 mg/ml in olive oil. Furosemide (Sigma) was dissolved at 0.4 mg/ml in 0.9% NaCl containing 0.01 N NaOH. We dissolved 4α-phorbol-12,13-didecanoate (4αPD; Sigma) at 10 μM in dimethylsulfoxide and added it to a luminal perfusate or bathing solution at a final concentration of 50 μM. The final concentration of dimethylsulfoxide was 0.2%. Sodium green (Molecular Probe) was dissolved at 64 μM in a 10 μM TRIZMA HCl/base buffer adjusted at pH 7.5. FITC-inulin (Sigma) was added into the luminal perfusate at 10–100 μM. All solutions and suspensions, except for the stock solution, were prepared before use.

**Statistics.** Data are given as the means ± SE (n = no. of experiments). Statistical significance in the mean values was evaluated by either Student’s t-test or Tukey-Kramer’s multiple comparison test. P values < 0.05 were considered significant.

**RESULTS**

**In vitro ion transports in isolated CCDs.** An increase in the luminal flow rate augmented CTX-sensitive K⁺ conductance in the luminal membrane of rabbit CNTs (20) and CTX-sensitive K⁺ secretion from rabbit CCDs (27), accompanied by an increase in the cytoplasmic Ca²⁺ concentration ([Ca²⁺]ᵢ) (21, 28). These observations suggested that the TRPV4 channel might be built into the mechanism underlying the distal flow-dependent K⁺ secretion. Thus we investigated the role of the TRPV4 channel in K⁺ secretion in mouse CCDs with a similar
ROLE OF TRPV4 IN A FLOW-SENSING MECHANISM

Effects of 4αPDD on ion transports in CCDs. It is known that 4αPDD activates the TRPV4 channel (5, 26). Thus we examined the effects of 4αPDD on both K⁺ secretion and Na⁺ reabsorption in isolated CCDs. In a preliminary experiment (see Supplemental Fig. S1; the online version of this article contains supplemental data), 4αPDD dose dependently induced a rise in [Ca²⁺], in cortical renal tubule suspensions obtained from TRPV4⁺/⁺ but not in those obtained from TRPV4⁻/⁻. The [Ca²⁺] was almost fully increased at 50 μM 4αPDD. Therefore, we employed this dosage for the full activation of TRPV4. As shown in Fig. 2A, a luminal application of 50 μM 4αPDD significantly stimulated the K⁺ secretion in TRPV4⁺/⁺ CCDs at a faster luminal flow rate. However, K⁺ secretion was not completely stimulated by 4αPDD at

A

B

Fig. 1. Flow dependence of K⁺ secretion (A) and Na⁺ reabsorption (B) in cortical collecting ducts (CCDs) isolated from either TRPV4⁺/⁺ or TRPV4⁻/⁻ mice. Both the K⁺ and Na⁺ fluxes were simultaneously measured at the perfusion pressure of 0.7 kPa for a slower luminal flow rate (n = 7 in TRPV4⁺/⁺ CCDs, and n = 6 in TRPV4⁻/⁻ CCDs) and 1.2 kPa for a faster luminal flow rate (n = 10 in TRPV4⁺/⁺ CCDs, and n = 8 in TRPV4⁻/⁻ CCDs) using an in vitro microperfusion technique. TRPV4, transient receptor vaniloid-4. **Both K⁺ secretion and Na⁺ reabsorption at the faster luminal flow rate in TRPV4⁺/⁺ mice were significantly greater than the other 3 mean values according to Tukey-Kramer’s multiple comparison test.

technique. We measured both net K⁺ and Na⁺ transports in isolated CCDs perfused at a relatively slow or relatively fast luminal flow by applying perfusion pressure at 0.7 or 1.2 kPa, respectively. These measurements were conducted in the CCDs isolated from either TRPV4⁺/⁺ or TRPV4⁻/⁻ mice pretreated with DOCA, because the net fluxes of K⁺ and Na⁺ were too small for accurate measurements in mice without DOCA pretreatment, as reported in rat CCDs (24).

Both K⁺ secretion and Na⁺ reabsorption at faster luminal flow rate showed a significant increase of more than threefold compared with those at a slower luminal flow rate in the CCDs isolated from TRPV4⁺/⁺ mice (TRPV4⁺/⁺ CCDs) (Fig. 1). In contrast to this, no significant augmentation of K⁺ secretion and Na⁺ reabsorption was found in the CCDs isolated from TRPV4⁻/⁻ mice (TRPV4⁻/⁻ CCDs) as a result of an increase in the luminal flow rate. The flow-dependent transport of either K⁺ or Na⁺ was completely lost in the CCDs by disruption of the TRPV4 gene.
a faster flow rate in TRPV4−/− CCDs (Fig. 2B). No significant stimulation of K+ secretion by luminal 4aPDD was observed at a slower luminal flow rate in either TRPV4+/+ CCDs (Fig. 2A) or TRPV4−/− CCDs (Fig. 2B). Similar results were obtained in Na+ reabsorption, which was simultaneously measured with K+ secretion (Fig. 3). It should be noted that 4aPDD did not stimulate K+ and Na+ transports in the absence of the TRPV4 channel, even though this phorbol ester might not be specific to the TRPV4 channel.

The stimulatory effect of Na+ reabsorption was not reproduced when the 4aPDD was applied at the same concentration from the basolateral side on TRPV4+/+ CCDs (Fig. 4). A basolateral application of 4aPDD caused a statistically significant but much smaller increase in the K+ secretion in TRPV4+/+ CCDs than that caused by its luminal application. Thus the effects of basolateral 4aPDD on these ion transports were clearly different from those of luminal application.

Effects of CTX and amiloride on ion transports in CCD. Selective K+ channels, such as the maxi-K+ and renal outer medullary potassium (ROMK) channels (Kir1.1), mediate the K+ transport in CCDs (27), while selective Na+ channels sensitive to amiloride mediate the Na+ transport in CCDs (12). The nonselective TRPV4 channel, however, might mediate both the K+ and Na+ transports in the luminal membrane of mouse CCDs because both K+ secretion and Na+ reabsorption were simultaneously stimulated by either an increase of the luminal flow or a luminal application of 4aPDD in TRPV4+/+ but not in TRPV4−/− mice. Thus we examined the contribution of both maxi-K+ and amiloride-sensitive Na+ channels to the net K+ and Na+ transports in mouse CCDs in the following experiments.

As shown in Fig. 5A, a luminal application of 1 µM CTX reduced both K+ secretion and Na+ reabsorption in TRPV4+/+ CCDs at a faster luminal flow rate. Similarly, both K+ secretion and Na+ reabsorption were reduced by the application of 10 µM amiloride to the tubular lumen at a faster luminal flow rate (Fig. 5B). The reduced levels of both K+ secretion and Na+ reabsorption were similar to the levels of the flow-independent components of these ion transports (Fig. 1). The major flow-dependent transport pathways of K+ and Na+ in the luminal membrane of CCDs, therefore, are the maxi-K+ channel and the amiloride-sensitive Na+ channel, respectively. The TRPV4 channel itself might make a minor contribution to K+ secretion and Na+ reabsorption as a transport pathway for these ions in mouse CCDs.

Urinary K+ excretion in vivo. To disclose the physiological role of the TRPV4 channel in urinary K+ excretion in vivo, we compared K+ excretion in TRPV4+/+ and TRPV4−/− mice. In the control period, the urine flow rate was 37 ± 6 µl·min⁻¹·kg BW⁻¹ in TRPV4+/+ mice and 53 ± 10 µl·min⁻¹·kg BW⁻¹ in TRPV4−/− mice. These urine flow rates were similar to that (30–40 µl·min⁻¹·kg BW⁻¹) estimated from the daily urine volume in metabolic cage experiments (11). The urinary K+ excretion in this period did not show a significant difference in TRPV4+/+ and TRPV4−/− mice not receiving a pretreatment.
with DOCA (Fig. 6A). However, the amount of K+ excretion was significantly smaller (~20% smaller) in TRPV4+/− mice after the urine flow (1.273 ± 116 μl·min⁻¹·kg BW⁻¹ in TRPV4+/− mice, n = 8, vs. 1.242 ± 101 μl·min⁻¹·kg BW⁻¹ in TRPV4−/− mice, n = 9) was accelerated by an application of furosemide. Similar results were observed in both types of mice receiving a pretreatment with DOCA (Fig. 6B).

**DISCUSSION**

Sensing of tubular flow by luminal rather than basolateral TRPV4 channels. As has been suggested (7), TRPV4 channels are predominantly distributed in distal tubules. We performed our own immunohistological staining with the antibody against the COOH terminal of TRPV4 and found a predominantly basolateral distribution, similar to that reported in a previous study (22) (see Supplemental Fig. S2). Although apical TRPV4 channel was not detected using our antibody, we considered that functional protein might be present in the luminal membrane of renal distal tubule. The present consequences suggested that the TRPV4 channel in the luminal membrane sensed the flow rate of tubular fluid. The basolateral 4αPDD caused a very small, but statistically significant, increase in K+ secretion and no significant change in Na+ reabsorption. Thus the luminal TRPV4 channel is at least functionally active, although the cytoplasmic localization of the TRPV4 channel just underneath the luminal membrane, e.g., in the endoplasmic reticulum, cannot be ruled out. On the other hand, an increase in the luminal flow or the perfusion pressure might induce mechanical stress by morphological change and may primarily stimulate basolateral TRPV4, which stimulates K+ excretion into the lumen. The additional effect of basolateral 4αPDD on K+ excretion was small, while the additional effect of luminal 4αPDD on K+ excretion was still vivid at a high luminal flow rate. Thus the high flow rate may primarily stimulate the basolateral TRPV4 toward the maximum activation and then activate luminal TRPV4. The strict primary site for TRPV4 to sense the flow rate was thereby obscured and remained unsolved.

**Fig. 5.** Inhibitory actions of charybdotoxin (CTX; A) or amiloride (B) on K+ secretion and Na+ reabsorption in CCD isolated from TRPV4+/− mice. The net ion fluxes were measured at a faster luminal flow rate (9.1 ± 0.6 nl/min, n = 6) before and after a luminal application of 1 μM CTX (A). The net ion fluxes were also measured at a faster luminal flow rate (8.1 ± 0.2 nl/min, n = 10) before and after a luminal application of 10 μM amiloride (B). *P < 0.05, t-test.

**Fig. 6.** Urinary K+ excretions in TRPV4+/− (n = 9) and TRPV4−/− mice (n = 9) not receiving exogenous mineral corticoids (A) and in TRPV4+/− (n = 8) and TRPV4−/− mice (n = 9) receiving deoxycorticosterone acetate (DOCA; 2 mg·mouse⁻¹·day⁻¹ sc) for 6 days (B). In the furosemide period, mice were administered 2 mg/kg body wt furosemide (iv) to accelerate urine production. *Significant difference between TRPV4+/− and TRPV4−/− mice according to unpaired Student’s t-test.
Contribution of the TRPV4 channel to flow-dependent K⁺ secretion in CCDs. The flow-dependent increase in K⁺ secretion that we observed was accompanied by an increase in Na⁺ reabsorption in CCDs, which might be explained by the assumption that the nonselective TRPV4 channel worked as a dominant pathway for the flow-dependent transports of K⁺ and Na⁺ ions. This alternative explanation, however, could be ruled out because a luminal application of either CTX or amiloride inhibited the flow-dependent increments of both K⁺ and Na⁺ transports. The inhibition of the maxi-K⁺ channel by CTX suppressed the flow-dependent component of K⁺ secretion and should depolarize the luminal membrane to reduce the driving force of Na⁺ reabsorption. The inhibition of Na⁺ channels by amiloride suppressed Na⁺ reabsorption and should hyperpolarize the luminal membrane to reduce the driving force of K⁺ secretion. In contrast to our observation, neither iberiotoxin (28) nor CTX (27) reduced the Na⁺ reabsorption in rabbit CCDs. Although the reason for this discrepancy is unclear, the expression of the Kir1.1 channel might be more abundant in rabbit CCDs than in mouse CCDs. The inhibition of the maxi-K⁺ channel might depolarize the luminal membrane of rabbit CCDs less than that of mouse CCDs. In either case, it is possible that the activation of the TRPV4 channel in response to an increase in the luminal flow stimulated the maxi-K⁺ channel to increase K⁺ secretion, which increases the driving force of Na⁺ reabsorption because of the hyperpolarization of the luminal membrane.

There was no significant difference between urinary K⁺ excretions in TRPV4+/+ and TRPV4−/− mice in the control period in Fig. 6. On the basis of the single nephron glomerular filtration rate (~8 nl/min) in mice (15), the luminal flow rate in mouse CCDs could be estimated as 1 nl/min or less under normal conditions. Because the urine flow rate in the control period in Fig. 6 was similar to that obtained from the daily urine volume, the luminal flow rate in CCDs should be 1 nl/min or less in this period. Thus the flow-dependent component of K⁺ secretion in CCDs should be minimal. On the other hand, urinary K⁺ excretion was significantly smaller (20% smaller) in TRPV4−/− than in TRPV4+/+ mice when an application of furosemide accelerated the urine flow rate 20–30 times. These results agreed well with those obtained from in vitro microperfusion experiments in both TRPV4+/+ and TRPV4−/− CCDs.

Sensing mechanism of the luminal flow in the K⁺ secretion. As discussed above, the TRPV4 channel plays an essential role in flow-dependent K⁺ secretion to stimulate the maxi-K⁺ channel. The luminal Ca²⁺ influx via the TRPV4 channel activated by an increase in the luminal flow seems to elicit a series of cellular reactions in CCDs. Namely, elevated [Ca²⁺]i activates the maxi-K⁺ channel to increase K⁺ secretion, resulting in the hyperpolarization of the luminal membrane to increase the driving force of Na⁺ reabsorption in the CCDs.

On the other hand, the luminal maxi-K⁺ channel showed Ca²⁺-independent stretch activation in intercalated cells (IC cells) of rabbit CCDs (13). If flow-dependent K⁺ secretion is mediated by the Ca²⁺-independent stretch activation of the maxi-K⁺ channel, the K⁺ ions should be flow dependently secreted in mouse CCDs in the presence of absence of the TRPV4 channel. However, we demonstrated in the present study that the flow-dependent component of K⁺ secretion completely disappeared in the TRPV4−/− CCDs. Thus it is likely that the change in the luminal flow is sensed by the TRPV4 channel but not by the maxi-K⁺ channel.

It was reported that an increase in the luminal flow elevated [Ca²⁺]i in both principal (PC) and IC cells in rabbit CCDs toward the level of 0.3–0.4 μM, although the source of Ca²⁺ was equivocal (28). The elevated level of [Ca²⁺]i was insufficient to activate the maxi-K⁺ channel because it was activated by >1 μM Ca²⁺ at negative membrane voltages (13). On the other hand, it is possible that even a small amount of luminal Ca²⁺ influx could increase the [Ca²⁺]i in the local region just underneath the luminal membrane enough to activate the maxi-K⁺ channel, as proposed in rabbit CNTs (20), even though the average [Ca²⁺]i was 0.3–0.4 μM. The expression of the TRPV4 channel can be detected in the luminal membrane of renal tubules (3). Therefore, the flow-sensing mechanism in the CCDs can be explained by assuming that there is luminal expression of the TRPV4 channel. This possibility should be clarified by using isolated membrane patches in the future in TRPV4+/+ and TRPV4−/− renal tubules.

Sensitivities of K⁺ secretion to temperature, mechanical stress, and phorbol ester. The TRPV4 channel was activated by an increase of the temperature of >28°C (6). In our experiments, which were performed at room temperature, the TRPV4 channel was not fully activated because both K⁺ secretion and Na⁺ reabsorption in TRPV4+/+ CCDs were poorly stimulated by 4αPDD at a low flow rate. They were stimulated more strongly at a high flow rate.

Thus preactivation of the TRPV4 channel by luminal flow-induced shear stress underlies the 4αPDD-induced stimulation of the TRPV4 channel in mouse CCDs. This observation suggests that physical stimuli, such as shear stress and heat, are more fundamental activators of the TRPV4 channel in the renal tubules than chemical stimuli. This idea may be supported by a similar observation that PTH stimulated the luminal Ca²⁺ influx in rabbit CNTs perfused in vitro at higher pressure (1.2 kPa) but not in those perfused at lower pressure (0.2 kPa) (21). In fact, stretch-activated cation channels found in the luminal membrane of rabbit CNTs were not stimulated by the membrane-permeable analog of cAMP, a second messenger of PTH, but the cation channels preactivated by the membrane stretch were highly stimulated by the cAMP analog in cell-attached patch-clamp experiments (21).

In addition to shear stress, viscosity is also thought to play a role in the stimulation of the TRPV4 channel in the flow-dependent mechanism (1). Because luminal fluid in the distal nephron does not contain viscous materials under normal conditions, we did not test the effect of viscosity. However, viscosity is increased in the nephrotic syndrome, and the TRPV4 channel may play a role in K⁺ loss in that disease.

We conclude that the TRPV4 channel in the luminal or the basolateral membrane plays a cardinal role in sensing the luminal flow in CCDs of the kidney. The activation of the TRPV4 channel allows Ca²⁺ influx, which results in the activation of the maxi-K⁺ channel and leads to the secretion of K⁺ into the luminal fluid. This mechanism is important in the excretion of the total amount of K⁺ in vivo (4, 9, 16).

**NOTE ADDED IN PROOF**

Recently, the contribution of TRPV4 to epithelial permeability was suggested in mammary gland cell monolayers (FASEB J 20: 1802–1812, 2006). Thus, the data for transepithelial voltage difference (Vᵣ)
in this experiment are important for the knowledge of permeability in the renal collecting duct. The results for $V_T$ before and after perfusion by different flow rates are shown below. The magnitude of negative $V_T$ in TRPV4$^{-/-}$ with a lower flow rate is significantly smaller than that in TRPV4$^{+/+}$ ($n = 12$, unpaired t-test). $V_T$ is diminished by an increase in transcellular Na$^+$ transport in TRPV4$^{+/+}$, but not in TRPV4$^{-/-}$. The $V_T$ in TRPV4$^{+/+}$ is not changed by application of 4ePDD to either side, because the change in $V_T$ by the activation of TRPV4 requires a longer period (FASEB J 20: 1802–1812, 2006) than the time for the experiment with this perfusion. Therefore, our results support that TRPV4 plays a role in transepithelial permeability suggested in mammary monolayers and may be compatible with the hypothesis that TRPV4 contributes to the Cl$^-$ shunt in Gordon’s syndrome (Am J Physiol Renal Physiol 290: F1303–F1304, 2006).

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