Epithelial Na⁺ channels are regulated by flow

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Epithelial Na⁺ channels are regulated by flow. Am J Physiol Renal Physiol 280: F1010–F1018, 2001.—Na⁺ absorption in the renal cortical collecting duct (CCD) is mediated by apical epithelial Na⁺ channels (ENaCs). The CCD is subject to continuous variations in intraluminal flow rate that we speculate alters hydrostatic pressure, membrane stretch, and shear stress. Although ENaCs share limited sequence homology with putative mechanosensitive ion channels in Caenorhabditis elegans, controversy exists as to whether ENaCs are regulated by biomechanical forces. We examined the effect of varying the rate of fluid flow on whole cell Na⁺ currents (I Na) in oocytes expressing α,β,γ-ENaC (mENaC) and on net Na⁺ absorption in microperfused rabbit CCDs. Oocytes injected with mENaC but not water responded to the initiation of superfusate flow (to 4–6 ml/min) with a reversible threefold stimulation of I Na without a change in reversal potential. The increase in I Na was variable among oocytes. CCDs responded to a threefold increase in rate of luminal flow with a twofold increase in the rate of net Na⁺ absorption. An increase in luminal viscosity achieved by addition of 5% dextran to the luminal perfusate did not alter the rate of net Na⁺ absorption, suggesting that shear stress does not influence Na⁺ transport in the CCD. In sum, our data suggest that flow stimulation of ENaC activity and Na⁺ absorption is mediated by an increase in hydrostatic pressure and/or membrane stretch. We propose that intraluminal flow rate may be an important regulator of channel activity in the CCD.

THE MAMMALIAN KIDNEY FILTERS large quantities of Na⁺ on a daily basis. Filtered Na⁺ is almost entirely reabsorbed within the nephron by a variety of distinct Na⁺-selective transport proteins expressed in the apical plasma membranes of renal tubular epithelial cells. The mammalian cortical collecting duct (CCD) is a major regulatory site of renal Na⁺ reabsorption (36, 46, 48, 53). Transcellular Na⁺ transport in this segment requires Na⁺ entry across the apical membrane of principal cells through amiloride-sensitive epithelial Na⁺ channels (ENaCs) (40, 45, 47) and its electronegic extrusion at the basolateral membrane by the Na⁺-K⁺ pump, K⁺, which accumulates in high concentration within the cell due to Na⁺-K⁺-ATPase-mediated basolateral exchange of K⁺ for Na⁺, passively diffuses from the cell into the tubular fluid through apical secretory K⁺ (SK) channels (17, 20, 60). The magnitude of K⁺ secretion is determined by its electrochemical gradient and by the permeability of the membrane to K⁺. ROMK, a member of the family of inwardly rectifying K⁺ channels that is expressed in the mammalian CCD (7, 23, 64, 65), is considered to represent the major functional subunit of the SK channel. This cell model predicts that transepithelial Na⁺ absorption in the CCD is a major determinant of the rate of K⁺ secretion.

ENaC, initially cloned from rat colon, exists as a multimeric complex comprised of homologous α-, β-, and γ-subunits (6, 8). ENaCs are localized to the apical membrane of epithelial cells not only of colon and distal nephron but also of airway and ducts of several secretory glands, where they constitute the rate-limiting step for Na⁺ reabsorption (45). Their activity is regulated by a variety of intracellular ions (Na⁺, Ca²⁺, H⁺) (4, 9, 41); selected kinases (protein kinases A and C, and sgk) (3, 11, 27); the ubiquitin ligase NEDD4 (21, 51); extracellular factors (aldosterone, arginine vasopressin, insulin, proteases) (31, 33, 37, 59); and other integral membrane proteins (i.e., CFTR) (44, 51, 55). The observation that ENaC subunits show structural homology to a family of Caenorhabditis elegans degenerin proteins, including mec-4, mec-10, and deg-1, proposed to form mechanosensory ion channels (18, 24, 25), has led to the speculation that ENaC is sensitive to membrane stretch.

Studies directed at examining the mechanosensitivity of ENaC have produced conflicting results. Palmer and Frindt (42) showed that negative hydrostatic pressure applied to the patch-clamp pipette led to a reversible increase in open probability (P o) of native apical Na⁺ channels in rat CCD in 6 of 22 patches. However, the majority of patches (15 of 22) showed no response to an increase in transmembrane pressure. These investigators suggested that the inconsistent response of ENaC to stretch and/or pressure reflected variability in the mechanical deformation of the apical membrane within the tip of the pipette.

The mechanosensitivity of ENaC has also been examined in nonepithelial expression systems. ENaC

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α-subunits expressed in mouse fibroblasts were activated in response to increases in negative hydrostatic pressure applied to the patch-clamp pipettes (32). α,β,γ-ENaC expressed in lipid bilayers was activated when a hydrostatic pressure gradient was applied across the bilayer (26). *Xenopus laevis* oocytes expressing α,β,γ-ENaC responded to cell swelling with either no change (5) or a decrease (29) in Na⁺ conductance and responded to cell shrinkage with an increase (29) or decrease (5) in Na⁺ conductance. Achard et al. (1) demonstrated in human B lymphocytes that a modest increase in the hydrostatic pressure of the solution bathing the cells activated an amiloride-sensitive Na⁺ channel, a response requiring an intact cytoskeleton. Furthermore, in that study, membrane stretch altered the amiloride sensitivity, cation selectivity, and inward-rectifying behavior of this channel when studied in the whole cell, patch-clamp configuration. Given the differences among the experimental systems and protocols used to elicit changes in transmembrane pressure, and the presumed variability in relative abundance of ENaC subunits and associated proteins expressed in each system, it is not unexpected that discrepant results have been reported.

The physiological relevance of the mechanosensitivity of ENaC becomes clearly apparent in the mammalian CCD, a nephron segment subject to continuous variations in rates of tubular flow. We examined the effect of increases in the rate of fluid flow on exogenous ENaC channels expressed in *X. laevis* oocytes and endogenous amiloride-sensitive Na⁺ channels in rabbit CCDs. Our results suggest that ENaC is a flow-regulated ion channel.

**METHODS**

**Reagents.** All chemicals were from Sigma (St. Louis, MO) unless stated otherwise.

Oocyte expression. We previously cloned and characterized mouse α,β,γ-ENaC (mENaC) cDNAs (2). cDNA for ROMK1 was a gift from S. Hebert (Vanderbilt University). cRNAs for wild-type α-, β-, and γ-mENaC subunits were synthesized with T3 RNA polymerase (Ambion, Austin, TX). cRNA for ROMK1 was synthesized with T7 RNA polymerase (Ambion). Stage V-VI *X. laevis* oocytes pretreated with 2 mg/ml type IV collagenase were injected with 4 ng of cRNA of each mENaC subunit or 4 ng of ROMK1 cRNA in 50 nl of H₂O. After injection, oocytes were incubated at 18°C in modified Barth’s saline (MBS; Table 1) containing 10 μg/ml sodium penicillin, 10 μg/ml streptomycin sulfate, and 100 μg/ml gentamicin sulfate, pH 7.2.

Two-electrode voltage clamp was performed 20–72 h after cRNA injection at room temperature (22–25°C) using a DigiData 1200 interface (Axon Instruments, Foster City, CA) and a TEV 200 voltage-clamp amplifier (Dagan, Minneapolis, MN), as previously described (50). Data acquisition and analyses were performed using pClAMP 6.03 software (Axon Instruments) on a 120-MHz Pentium PC (Gateway 2000, N. Sioux City, SD). Pipettes were pulled from borosilicate glass capillaries (World Precision Instruments, Sarasota, FL) with a micropipette puller (Sutter Instrument, Novato, CA) and had resistances of 0.5–5 MΩ when filled with 3 M KCl and inserted into the bath solution.

Oocytes were placed in the center of a flat 2-cm-diameter recording chamber containing 1 ml of bath solution. Whole cell currents were measured at −100 mV 800 ms after initiation of the voltage clamp. Amiloride-sensitive currents were defined as the current difference between that measured in the absence and presence of amiloride (0.1 or 1 mM, as indicated) in the bath solution. Ba²⁺-sensitive currents were defined as the current difference between that measured in the absence and presence of 10 mM BaCl₂ in the bath solution.

Two bath solutions were used to study currents in oocytes (Table 1). A Na⁺ gluconate solution was utilized to examine the effects of fluid flow on α,β,γ-mENaC. BaCl₂ (5 mM) was added to this solution to inhibit background endogenous K⁺ channel currents in oocytes. A Na⁺/K⁺ chloride solution was used to compare the effects of fluid flow on oocytes expressing mENaC or ROMK1. Oocytes were transferred from MBS to the bath solution before a final transfer to the recording chamber. The volume of the oocyte chamber was maintained constant by vacuum aspiration of fluid when the chamber volume exceeded 1 ml.

### Table 1. Composition of solutions

<table>
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<tr>
<th></th>
<th>Modified Barth’s Saline</th>
<th>Na⁺ Gluconate</th>
<th>Na⁺/K⁺ Chloride</th>
<th>Burg’s</th>
<th>HEPES-Buffered</th>
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Composition of solutions is expressed in mM.
To examine the effect of fluid flow on macroscopic mENaC currents, inward Na\(^{+}\) currents were measured at a testing potential of \(-100\) mV initially without application of flow and then at 1, 2, 3, 4, and 5 min after initiation of superfuse flow at a rate of 4–6 ml/min, adjusted by gravity. Oocytes were then perfused with solutions containing amiloride or Ba\(^{2+}\) to determine the amiloride- or Ba\(^{2+}\)-insensitive components of the whole cell current, respectively. Maximum amiloride-sensitive Na\(^{+}\) currents (mENaC) or Ba\(^{2+}\)-sensitive currents (ROMK1) measured in the presence of fluid flow were normalized to the basal currents in the absence of flow.

**Measurement of cation transport in microperfused CCDs.** Adult female New Zealand White rabbits were obtained from Charles River (Quebec, ON) or Covance (Denver, PA). A single CCD was microdissected from each animal, transferred immediately to a temperature and O\(_2\)-CO\(_2\)-controlled specimen chamber, and mounted on concentric glass pipettes, as previously described (46). Segments were perfused and bathed at 37°C in either Burg’s solution or, to measure transport in the presence of Ba\(^{2+}\), a HEPES-buffered solution (61) (Table 1). Both solutions were adjusted to 290 ± 2 mosmol/kgH\(_2\)O. Because there were no transepithelial osmotic gradients, water transport was assumed to be zero (46).

Burg’s solution was bubbled with and then continuously suffused with 95% O\(_2\)-5% CO\(_2\) to maintain pH at 7.4 at 37°C. To examine whether differences in luminal Na\(^{+}\) concentration and/or flow affect the magnitude of Na\(^{+}\) backflux, the rate of “bath-to-lumen” Na\(^{+}\) transport was measured in several CCDs perfused with a 0-Na\(^{+}\) solution (Table 1) (260 mosmol/kgH\(_2\)O) (52) containing 0.1 mM amiloride to inhibit the lumen-to-bath ENaC-mediated Na\(^{+}\) absorptive flux. In all microperfusion studies, samples of tubular fluid were collected under water-saturated light mineral oil by timed filling of a precalibrated 20-nl volumetric constriction pipette. Flow rate was varied by adjusting the height of the perfusate reservoir.

To study flow-dependent transport, the sequence of flow rates was randomized within each group of tubules to minimize any bias induced by time-dependent changes in transport. In general, three to four timed collections of tubular fluid were made at each of two to three flow rates in a given segment. The concentrations of Na\(^{+}\) and K\(^{+}\) in the samples of collected tubular fluid were measured by helium glow photometry, and the mean rate of ion transport (in pmol-min\(^{-1}\)-mm tubular length\(^{-1}\)) at each flow rate was calculated as previously described (46). Net absorption is denoted as a positive transport rate and net secretion as a negative value. Flow rate was varied at least twofold in each segment. To determine the concentrations of Na\(^{+}\) and K\(^{+}\) delivered into the tubular lumen, three to four samples of tubular fluid were collected at the end of each experiment after addition of 100–200 μM ouabain to the bathing solution.

The transepithelial voltage (V\(_{ce}\)) was measured between symmetrical calomel electrodes continuous with the perfusion pipette via a 0.16 M NaCl-agarose bridge and referenced to the bath, as previously described (46). Readings were taken at the midpoint of each collection of tubular fluid and averaged.

The change in viscosity of Burg’s solution induced by addition of 5% dextran was measured using a falling-ball viscometer (no. 1, Gilmont Instruments, Barnant, Barrington, IL).

**Statistics.** Data are expressed as means ± SE; n equals the number of oocytes or tubules. Significant differences were determined by paired t-test, analysis of variance, or linear regression analysis, as appropriate. Significance was asserted if \(P < 0.05\).

**RESULTS**

Flow activates whole cell Na\(^{+}\) currents in oocytes expressing α,β,γ-mENaC. X. laevis oocytes injected with α-, β-, and γ-mENaC cRNA were examined by using a two-electrode voltage clamp at \(-100\) mV in the presence and absence of perfusion with the Na\(^{+}\) gluconate solution. In the absence of perfusion (i.e., no flow), the whole cell Na\(^{+}\) current (I\(_{Na}\)) was 10.0 ± 1.6 μA (n = 55; Fig. 1, A and C). Initiating bath flow from 0 (no flow) to the range of 4–6 ml/min led to a 3.3 ± 0.4-fold increase in amiloride-sensitive I\(_{Na}\) (n = 55; \(P < 0.001\); Fig. 1, A and C). Amiloride-sensitive Na\(^{+}\) currents were not observed in water-injected oocytes either in the absence or presence of flow (n = 10; Fig. 1B). The maximal increase in I\(_{Na}\) in response to flow was quite variable (Fig. 1D), and in 12 of 55 oocytes the increase was <40%. Given the variability in the flow response, it was difficult to establish a dose-response relationship (i.e., flow rate vs. I\(_{Na}\)) with the oocyte expression system. Flow-mediated increases in I\(_{Na}\) were observed within 1 min after initiation of oocyte perfusion and reached a plateau within 3–5 min (Fig. 1E). Flow activation of I\(_{Na}\) was reversible, as I\(_{Na}\) fell when the flow was stopped. Flow activation of I\(_{Na}\) was not associated with a significant change in the resting membrane potential of the oocyte (Fig. 1F). The reversal potential of oocytes expressing mENaC was 2.1 ± 0.9 mV in the absence of flow and 0.3 ± 0.9 mV in the presence of flow (n = 20; \(P = 0.18\)). These data suggest that the Na\(^{+}\) concentration gradient across the oocytes was not appreciably altered by flow.

ROMK1 is an inwardly rectifying K\(^{+}\) channel and, like ENaC, is expressed on the apical plasma membrane of collecting duct principal cells. To examine whether ROMK1 is flow activated, oocytes from a single batch were injected with either α,β,γ-mENaC or ROMK1 cRNAs. Whole cell currents were measured at a holding potential of \(-100\) mV before and 1–2 min after the flow of the Na\(^{+}\)/K\(^{+}\) chloride bath was initiated at a rate of 5–6 ml/min. The specificity of the flow-activated currents was determined by the subsequent addition of amiloride (for ENaC) or BaCl\(_2\) (for ROMK). I\(_{Na}\) in oocytes expressing α,β,γ-mENaC increased 6.3 ± 1.8-fold in response to flow (n = 8; \(P < 0.001\)), whereas whole cell K\(^{+}\) currents in oocytes expressing ROMK1 increased by only 1.7 ± 0.2-fold (n = 8; \(P < 0.02\)) (Fig. 2).

Flow activates Na\(^{+}\) absorption in rabbit CCD. To examine the effect of flow rate on net Na\(^{+}\) absorption in the rabbit CCD, single CCDs were perfused at increasing flow rates and the rates of net Na\(^{+}\) absorption were measured. The relationship between flow rate and net Na\(^{+}\) absorption was plotted for the rabbit distal tubule. The rate of net Na\(^{+}\) absorption increased significantly as flow rate was increased from 0.4 to 3 nl·min\(^{-1}·\)mm\(^{-1}\) (r = 0.97; \(P < 0.05\) by linear regression analysis), the physiological range of flow rates reported for the rabbit distal tubule.
nephron (12). \( V_n \) remained unchanged as flow rate was increased (\( P = 0.26; \) Table 2). As reported previously by ourselves (46) and others (15, 22, 35, 52), net K\(^+\) secretion in the CCD was also stimulated by increasing flow rate (Table 2). In agreement with previous observations (38, 54), 0.1 mM amiloride added to the luminal perfusate (\( n = 4 \)) significantly inhibited net Na\(^+\) absorption compared with transport rates measured at comparable flow rates in the absence of the inhibitor (\( P < 0.03; \) Fig. 3).

Net Na\(^+\) absorption in the CCD reflects the sum of opposing processes of Na\(^+\) absorption and secretion (52). To determine whether a flow-induced reduction in bath-to-lumen Na\(^+\) backflux into the lumen could account for the flow stimulation of net Na\(^+\) absorption, net Na\(^+\) transport was measured in a group of CCDs perfused with a slightly hypotonic 0-Na\(^+\) perfusate containing amiloride and bathed in Burg’s solution. These conditions were selected to inhibit lumen-to-bath and maximize bath-to-lumen transepithelial Na\(^+\) transport. An increase in flow rate from 0.9 ± 0.1 to 2.7 ± 0.4 nl·min\(^{-1} \) ·mm\(^{-1} \) led to no significant change in the rate of net Na\(^+\) transport (\( -7.4 ± 3.4 \) vs. \( -6.5 ± 5.7 \) pmol·min\(^{-1} \) ·mm\(^{-1} \); \( n = 4; P = 0.05 \) in CCDs perfused under these conditions.

Within the CCD, Na\(^+\) absorption is closely coupled to K\(^+\) secretion. To examine whether activation of apical K\(^+\)-conductive pathways at high tubular fluid flow rates, leading to hyperpolarization of the apical membrane potential, contributes to the flow dependence of net Na\(^+\) absorption, several CCDs were perfused in the presence of the K\(^+\) channel inhibitors Ba\(^{2+}\) (4 mM) and tetraethylammonium (TEA; 5 mM). Whereas Ba\(^{2+}\) is a relatively nonspecific inhibitor of K\(^+\) channels, TEA blocks the maxi-K\(^+\) channel (16), an apical K\(^+\) channel that is rarely open at the physiological membrane potential but is activated by depolarization, membrane stretch, and an increase in intracellular Ca\(^{2+}\) concentration (16, 39).

The effect of K\(^+\) channel inhibitors on transport was measured in CCDs perfused and bathed in the HEPES-buffered solution containing no HCO\(_3\) or phosphate,
the latter reagents removed due to the low solubility of BaHPO₄. There was no significant difference observed between the rates of net Na⁺ transport measured in Burg’s solution (17.4 ± 3.9 and 38.1 ± 3.5 pmol·min⁻¹·mm⁻¹) at flow rates of 1 and 3 nl·min⁻¹·mm⁻¹, respectively; n = 7) and the HEPES-buffered solution (20.4 ± 3.7 and 39.3 ± 3.6 pmol·min⁻¹·mm⁻¹ at similar flow rates; n = 5). In contrast, the rates of net K⁺ secretion in Burg’s solution (−16.3 ± 2.2 and −34.0 ± 5.9 pmol·min⁻¹·mm⁻¹ at flow rates of 1 and 3 nl·min⁻¹·mm⁻¹, respectively; n = 7) exceeded those measured in the HCO₃⁻-free HEPES-buffered solution (−8.0 ± 1.9 and −14.3 ± 2.4 pmol·min⁻¹·mm⁻¹ at similar flow rates; n = 5) (P < 0.03). Sharège and Stoner (49) first demonstrated that removal of HCO₃⁻ from the perfusate led to a 50% reduction in net K⁺ secretion in CCDs initially bathed in Burg’s solution without affecting net Na⁺ absorption, an effect they attributed to luminal acidification by the entry of CO₂. The stimulation of K⁺ secretion (−8.0 ± 1.9 to −14.3 ± 2.4 pmol·min⁻¹·mm⁻¹; n = 5; P < 0.01) elicited by an increase in tubular fluid flow rate from 1 to 3 nl·min⁻¹·mm⁻¹ was completely blocked by luminal addition of Ba²⁺/TEA (−4.2 ± 0.7 to −3.5 ± 1.3 pmol·min⁻¹·mm⁻¹; n = 6; P = NS) (Fig. 4D). In contrast, the twofold stimulation of net Na⁺ absorption (20.4 ± 3.7 to 39.3 ± 3.6 pmol·min⁻¹·mm⁻¹; n = 5; P < 0.03) elicited by an increase in tubular fluid flow rate from 1 to 3 nl·min⁻¹·mm⁻¹ was unaffected by inhibition of K⁺ secretion (16.8 ± 4.4 to 49.0 ± 15.0 pmol·min⁻¹·mm⁻¹; n = 6; P < 0.05) (Fig. 4A). Increasing the rate of luminal perfusion did not significantly alter Vₑ (−8.2 ± 2.6 to −6.6 ± 1.5 mV; n = 5), even in the presence of luminal K⁺ channel inhibitors (−4.2 ± 1.0 to −4.5 ± 1.2 mV; n = 6).

Within the vasculature, mechanical forces, including shear stress and the dragging frictional force created by blood flow, modulate endothelial cell function. Shear stress is determined by both the viscosity and the velocity of fluid flow (13). With the assumption that the diameter of the CCD remains unchanged at a constant flow rate, an increase in shear stress is expected to be directly proportional to an increase in viscosity (62). To determine whether the flow-induced stimulation of transepithelial Na⁺ transport in the CCD was related to an alteration in the frictional forces (shear stress) to which the apical surfaces of epithelial cells were exposed, net Na⁺ absorption was measured in a group of CCDs perfused at a constant flow rate (1.5 ± 0.2 nl·min⁻¹·mm⁻¹) before and after elevation of luminal viscosity. The 5.5-fold increase in luminal viscosity achieved by addition of 5% dextran (mean molecular mass 413,000, Sigma) to the luminal perfusate did not alter the rate of net Na⁺ absorption (18.2 ± 2.1 vs. 17.8 ± 2.3 pmol·min⁻¹·mm⁻¹; n = 6; P = NS) (Fig. 5). Nor did the presence of luminal dextran affect the rate of net K⁺ secretion in this group of CCDs (−9.3 ± 2.3 vs. −10.3 ± 3.2 pmol·min⁻¹·mm⁻¹; P = NS) (Fig. 5).

**DISCUSSION**

Flow rates within the distal nephron, including the cortical collecting duct, increase in response to expansion of the extracellular fluid volume or administration of diuretics and fall in response to volume depletion (20). These clinical observations led us to speculate that flow-induced changes in hydrostatic pressure, cell membrane stretch, or fluid shear stress might directly or indirectly regulate activity of apical ion channels in microperfused rabbit cortical collecting ducts (CCDs). The rates of net Na⁺ absorption (pmol·min⁻¹·mm tubular length⁻¹) were measured in single CCDs micropерfused in vitro at 2–3 flow rates in the absence (●, solid line) or presence (○, dotted line; n = 4) of 0.1 mM amiloride. Values are means ± SE. For CCDs perfused with Burg’s solution in the absence of amiloride, each data point represents the average rate of transport measured in 6–13 tubules. In the absence of amiloride, the rate of net Na⁺ absorption increased with faster flow rates (r² = 0.97 by linear regression analysis; P < 0.05). Luminal amiloride significantly inhibited net Na⁺ absorption at the two flow rates studied (P < 0.03 compared with Burg’s solution alone).
this nephron segment. Given the importance of the collecting duct in Na⁺ absorption and the evidence suggesting that ENaC may be a mechanosensitive ion channel, we sought to examine whether ENaC activity and Na⁺ transport are regulated by fluid flow rate.

Our results show that amiloride-sensitive Na⁺ currents in X. laevis oocytes expressing ENaCs are flow activated (Fig. 1) in a reversible manner. The response to flow was quite variable as we observed little or no response to changes in flow rates in some experiments (Fig. 1D). We speculate that this variability in response may be due, at least in part, to the high baseline variability in ENaC Pₐ that has been previously reported in rat principal cells (42) and M-1 mouse collecting duct cells (10).

Mechanosensitive channels in the worm C. elegans are tethered to both extracellular and intracellular proteins (56), and these interactions are thought to be required for mechanotransduction. Oocytes expressing ENaCs are surrounded by a vitelline membrane that could serve a similar purpose in the variable mechanosensory response of ENaC expressed in oocytes. We have observed that oocytes stripped of their vitelline membrane are too fragile for perfusion experiments to examine whether there is retention or loss of flow activation of ENaC.

In microperfused rabbit CCDs, an increase in tubular fluid flow rate within the physiological range is associated with an increase in amiloride-inhibitable net transepithelial Na⁺ absorption (Figs. 3 and 4). This flow-induced response was not due to a reduction in Na⁺ backflux into the lumen at high flow rates. Furthermore, flow-stimulated Na⁺ absorption can be dissociated from net K⁺ secretion and is not accompanied by an increase in Vₑ. The absence of flow-induced change in Vₑ, even under conditions where apical K⁺ secretion was inhibited, may reflect an increase in the paracellular permeability to Cl⁻, leading to movement of negative charge out of the lumen.

In contrast to the more threefold increase in amiloride-sensitive Na⁺ currents induced by flow over oocytes expressing ENaC, flow led to only a modest increase in K⁺ currents in oocytes injected with ROMK cRNA (Fig. 2). The low sensitivity of ROMK to flow is compatible with parallel observations in isolated perfused CCDs showing that flow-induced stimulation of net K⁺ secretion in isolated perfused rabbit CCDs is blocked by TEA (63), an inhibitor of maxi-K but not SK or ROMK channels (16, 23, 60, 64). These data suggest that flow-dependent K⁺ secretion is mediated by a maxi-K channel whereas baseline K⁺ secretion is mediated by the SK/ROMK channel.

It is well established that NaHCO₃ reabsorption in the proximal tubule increases in response to an increase in axial flow rate due to flow-dependent stimulation of Na⁺/H⁺ antiporter activity (43). Perfusion rate has also been shown to modulate Na⁺/H⁺ exchange and the rate of H⁺ secretion in cultured opossum kidney cells (19). Flow dependence of solute reabsorption in the proximal tubule, with its apical brush-
border membrane, has been attributed to flow rate-induced alterations in concentration gradients in the vicinity of the apical membrane. However, using a mathematical modeling approach, Krahn and Weinstein (34) were unable to demonstrate that gradients accumulate in an unstirred layer at the apical membrane of the proximal tubule brush border. We do not consider that an unstirred layer effect contributes significantly to our observations. First, in the oocyte experiments, there was no significant difference between the reversal potential measured at zero and high flow rates. This suggests that the flow-induced increase in \( I_{Na} \) is not due to changes in the relative \( Na^+ \) concentration at the external face of the channel; i.e., imposition of high flow did not alter the \( Na^+ \) concentration in an unstirred layer at the cell membrane. Although the magnitude of unstirred layers in the intact CCD is unknown, we speculate that it is no greater than that in the proximal tubule. Furthermore, the maximal difference (20 mM; Table 2) between \( Na^+ \) concentrations of perfused (\( \sim 140 \) mM) and collected tubular fluids indicates that it is likely that \( Na^+ \) concentrations at the CCD plasma membrane were always well in excess of the Michaelis-Menten constant of ENaC for \( Na^+ \), recently reported to be 38 mM (30).

Mechanical forces regulate cell function in a variety of tissues but have been particularly well studied in endothelial cells (13, 57). Because of their location within the vasculature, endothelial cells experience three types of mechanical forces: hydrostatic pressure, circumferential stretch or tension, and fluid shear stress generated by the frictional force of blood (13, 57). Of these, shear stress may be particularly important because of its effects on cytoskeletal remodeling, release of growth factors and vasoactive substances, and changes in gene expression, cell metabolism, and cell morphology (13, 57). Although the signaling pathways by which physical forces are transduced into biochemical signals are actively under investigation, it is apparent that the mechanosensitive ion channel represents a common mechanism by which a variety of cells sense changes in mechanical stimuli. Several different mechanosensitive ion channels have been identified in endothelial cells, including a shear-responsive voltage-gated \( Na^+ \) channel (58) and \( K^+ \) channel and a stretch-activated \( Ca^{2+} \) channel (14, 28).

We propose that mechanical forces regulate the function of renal tubular cells, as they do in endothelial cells. Our observation that a 5.5-fold increase in luminal viscosity did not alter the rate of net \( Na^+ \) absorption in CCDs perfused at a constant flow rate (Fig. 5) suggests that flow-induced stimulation of \( Na^+ \) transport is not due to an alteration in the frictional forces (shear stress) to which the apical surfaces of epithelial cells were exposed. Whether flow stimulation of ENaC activity and transepithelial \( Na^+ \) absorption are due to an increase in hydrostatic pressure and/or membrane stretch remains to be explored. Also not addressed by the present studies is whether this represents a direct effect on the channel protein or an indirect effect transduced by an as yet unidentified signaling pathway.

Investigation of these questions represents an important goal toward understanding the regulation of renal epithelial cell function in the context of the physical forces that prevail in vivo.

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