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Regulation of glomerulotubular balance: flow-activated proximal tubule function

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Abstract

The purpose of this review is to summarize our knowledge and understanding of the physiological importance and the mechanisms underlying flow-activated proximal tubule transport. Since the earliest micropuncture studies of mammalian proximal tubule, it has been recognized that tubular flow is an important regulator of sodium, potassium, and acid-base transport in the kidney. Increased fluid flow stimulates Na^+ and HCO_3^- absorption in the proximal tubule via stimulation of Na/H-exchanger isoform 3 (NHE3) and H^+ -ATPase. In the proximal tubule, brush border microvilli are the major flow sensors, which experience changes in hydrodynamic drag and bending moment as luminal flow velocity changes and which transmit the force of altered flow to cytoskeletal structures within the cell. The signal to NHE3 depends upon the integrity of the actin cytoskeleton; the signal to the H^+ -ATPase depends upon microtubules. We have demonstrated that alterations in fluid drag impact tubule function by modulating ion transporter availability within the brush border membrane of the proximal tubule. Beyond that, there is evidence that transporter activity within the peritubular membrane is also modulated by luminal flow. Secondary messengers that regulate the flow-mediated tubule function have also been delineated. Dopamine blunts the responsiveness of proximal tubule transporters to changes in luminal flow velocity, while a DA1 antagonist increases flow sensitivity of solute reabsorption. IP3 receptor-mediated intracellular Ca^{2+} signaling is critical to transduction of microvillus drag. In this review, we summarize our findings of the regulatory mechanism of flow-mediated Na^+ and HCO_3^- transport in the proximal tubule and review available information about flow sensing and regulatory mechanism of glomerulotubular balance.

Keywords

Flow dependent; Kidney NaCl; HCO_3^- ; Transport; Flow sensors; Calcium signals

Introduction

Flow-modulated salt and water transport in proximal tubules has been recognized for more than four decades. This perfusion-absorption balance in the proximal tubule has been termed glomerulotubular balance (GTB) [51]. Historically, one point of difficulty was to determine whether axial flow directly or indirectly regulates tubule transport, specifically, why the GTB convincingly demonstrated by Schnermann et al. in vivo [38] could not also be demonstrated in vitro in single-perfused rabbit tubules by Burg and Orloff [10]. However, it is relatively clear that flow-stimulated K secretion in the collecting tubule, by the mechanism of flow-stimulated MaxiK channel activity, and flow-stimulated Ca^{2+} influx play a critical role for the regulation in the collecting tubule [30, 52]. Although many investigators examined the flow effect on the proximal tubule transport, the mechanism and regulation of GTB remained uncertain [49]. We undertook examination of the flow effect on Na and HCO_3^- absorption by isolated tubule perfusion of mouse proximal tubules as the first study [17]. In that work, we demonstrated that flow-modulated Na/H-exchanger isoform 3 (NHE3) activity is the basis for flow-dependent proximal tubule Na^+ reabsorption. Flow-stimulated NHE3 and H-ATPase activities both contribute to the increased HCO_3^- absorption by higher flow [17]. This perfusion-absorption balance is independent of neuronal and systemic hormonal regulation and requires the intact actin cytoskeleton to transmit the signal of altered axial flow sensed by brush border micro-villi [14]. Changes in tight junction permeabilities do not play a role in flow-activated sodium and bicarbonate transport [17]. We also developed a theory and an equation that enable us to calculate the changes of torque at the base of the brush border microvilli due to fluid drag forces on their tips. We were able to demonstrate that flow-induced changes in Na^+ and HCO_3^- absorption are torque dependent (bending moment at the apical membrane due to fluid flow) [14]. Our experimental data proved the hypothesis that brush border microvilli serve as the mechanosensors of axial flow along the proximal tubule [14]. By using our theory which takes into account the changes of tubular diameter with flow, we were able to resolve the long-standing mystery as to why the GTB demonstrated in vivo did not appear to be present in single-perfused rabbit proximal tubule [43] (vide infra). Our mathematical model and experimental data indicated that luminal flow also affects peritubular transporters, as the flow produces only minor changes in cell volume [50]. In the studies of mouse proximal tubule cells, we have shown that fluid shear stress stimulates NHE3 and H-ATPase trafficking to the apical and Na/K-ATPase to the basolateral membrane surfaces. The actin cytoskeleton reorganization contributes to the perfusion-absorption balance, and flow stimulates NHE3 and Na/K-ATPase trafficking [19]. This observation is captured by a mathematical model that shows that both apical and basolateral transporters are regulated by flow [50]. To understand the regulatory mechanisms of GTB, the following three major signaling transduction pathways have been examined: angiotensin II (Ang II), dopamine, and calcium signals [15, 16, 47]. It has been demonstrated that the AT1 receptor is important to maximize the NHE3 activity activated by flow; however, it is not critical for the flow-stimulated HCO_3^- transport, which still exists when the inhibitors are present or when the AT1a receptor is knocked out [15]. Dopamine, which stimulates NHE3 endocytosis via a PKA-dependent mechanism, has no effect on baseline fluxes but abrogates the flow-stimulated Na^+ and HCO_3^- absorption [18]. A comparison of the changes in torque and

changes in transport activity by flow showed that dopamine reduced, and blocking of the dopamine receptor increased the tubule sensitivity to microvillus torque. Thus, the tubule sensitivity changes in microvillus torque are dependent upon transporter availability on the membrane surface [18]. It was also demonstrated that changes in extracellular Ca^{2+} did not alter flow signals, but inositol 1,4,5-trisphosphate (IP3) receptor-mediated intracellular Ca^{2+} signaling is a critical step in transduction of microvillus drag to modulate Na^+ and HCO_3^- transport [16]. This recent work has been a start in a program to define the underlying cellular mechanisms and regulation of GTB.

Both NHE3 and H-ATPase are regulated by flow

Despite changes in glomerular filtration rate (GFR), the fraction of the filtrate fluid, sodium chloride, and bicarbonate reabsorbed from the proximal tubule remains essentially constant, and this is termed glomerular tubular balance (GTB) [38]. An increase of GFR increased salt and water absorption in the proximal tubule in rat and dog studied by renal clearance and free flow micropuncture in vivo [22, 23, 38]. In the early 80s, Chan, Liu and Cogan, and Alpern et al. reported flow activates not only Na^+ but also HCO_3^- absorption in the proximal tubule of rat kidney [1, 13, 28]. However, all this work was done in vivo, but flow dependence could not be demonstrated for proximal tubule transport in vitro [10]. We measured flow-mediated Na^+ and HCO_3^- absorption in isolated proximal tubules from the mouse kidney and found that increased perfusion rates significantly enhanced absorption of both volume (J_v) (or equivalently, Na^+ flux, J_{Na}) and HCO_3^- (J_{HCO_3}). Figure 1a displays the rate of Na^+ absorption in response to changing perfusion rate, and Fig. 1b is the flow-mediated HCO_3^- absorption. Both Na^+ and HCO_3^- absorption rates are proportionally increased with increased perfusion rates, $R^2 = 0.96$ and $R^2 = 0.99$, respectively, indicating that flow stimulated both Na and HCO_3^- absorption in vitro. Experimental data confirmed the previous demonstrations but also provided the first evidence that flow-dependent proximal tubule transport of Na^+ and HCO_3^- does not depend upon Starling forces, neuronal control, or systemic hormonal regulation.

NHE3 is the predominant isoform for apical membrane Na^+/H^+ exchange in the proximal tubule [45]. We have reported that Na^+ and HCO_3^- absorption was reduced by 60% in NHE3 knockout mice compared with wild-type animals [46] and that in the absence of NHE3, neither NHE2 nor any other EIPA-sensitive NHE isoform contributes to mediating HCO_3^- reabsorption in the proximal tubule [46]. A significant component of HCO_3^- reabsorption (via H^+ secretion) in the proximal tubule is mediated by bafilomycin-sensitive H^+ -ATPase [46]. Figure 1c, d displays the role of NHE3 and H-ATPase on flow-activated tubule transport. The flow-induced changes on Na^+ and HCO_3^- absorption were compared under conditions with and without blocking of NHE3 (by EIPA), H-ATPase (by bafilomycin), or both. EIPA and bafilomycin only partially blocked the flow effects on both J_v and J_{HCO_3} ; however, the flow-stimulated HCO_3^- absorption was completely abolished, indicating that both NHE3 and H-ATPase activity are regulated by tubular flow. The remaining flow-increased Na absorption when NHE3 is blocked suggests that flow also stimulates other Na^+ transport mechanisms besides NHE3.

NHE3, H-ATPase, and Na/K-ATPase trafficking and expressions are regulated by flow

Flow-dependent Na^+ and HCO_3^- reabsorption is due to modulation of both NHE3 and vacuolar H^+ -ATPase activities. It has been suggested that the intact actin cytoskeleton provides a structural framework for proximal tubule cells to transmit mechanical forces and subsequently modulate cellular function [17]. Thus, it was important to know whether membrane transporter protein trafficking is involved in fluid shear stress (FSS)-induced regulation of the transporter activity. This study was carried out on cultured immortalized mouse proximal tubule cells, and the NHE3, H-ATPase, and Na/K-ATPase localization and expressions were examined both in the absence and presence of 1-dyne/cm^2 FSS for 3 h. Figure 2 shows that FSS leads to an increment in the amount of protein expression and a translocation of apical NHE3 (Fig. 2a) and V-ATPase (Fig. 2b) from the intracellular compartment to the apical plasma membrane and Na^+/K^+ -ATPase (Fig. 2c) to the basolateral membrane. Disrupting actin by cytochalasin D blocks the FSS-induced changes in NHE3 and Na^+/K^+ -ATPase, but not V-ATPase (Fig. 2c). In contrast, FSS-induced V-ATPase redistribution and expression are largely inhibited by colchicine, an agent that blocks microtubule polymerization. These findings indicated that FSS-induced transporter trafficking was not only limited to the apical domain of the plasma membrane, where microvilli and primary cilium mechanotransducers make direct contact with the flow, but also involved basolateral membrane protein activities such as Na^+/K^+ -ATPase. The actin cytoskeleton disruption blocked the FSS-stimulating effect on upregulation and trafficking of NHE3 and Na^+/K^+ -ATPase, but an FSS-induced increase in apical V-ATPase abundance relies on an intact microtubule network, not actin filaments [19]. This study also provided evidence that peritubular transporters can be activated by luminal flow supporting the mathematical model of Weinstein et al. [50]. The peritubular transporters must be modulated by luminal flow to allow coordinated transport of Na^+ and HCO_3^- , at the basolateral membrane, and entry into the interstitium and peritubular capillaries from the cell when apical transporter activity is increased. In that regard, the possibility has been raised that changes in luminal drag may directly impact peritubular transporters by altering the forces on peritubular anchoring proteins and perhaps local Ca^{2+} channels [37].

Brush border microvillus is the major flow sensor in the proximal tubule

The brush border is a remarkable mechanosensing apparatus that involves ~4000 membrane-bound actin filament bundles (microvilli) that are equally spaced and nearly identical in height on each PT epithelial cell [32, 33]. Based on model calculations, Guo et al. first proposed that the brush border microvilli serve as the sensor for axial flow along the proximal tubule due to their homogeneity in height and spacing [24]. Such homogeneity would not be necessary if the primary function of the microvilli was solely to increase apical transport area. Furthermore, their model showed that the microvilli acted as stiff bristles since their central actin filament bundle hardly deflected at physiological flow rates in contrast to primary cilia. In their hypothesis, the drag force on each microvillus produces a torque (bending moment) on the actin filament bundle in the core of each microvillus that is transmitted to the actin cortical network in the underlying cytoskeleton. This hypothesis is

supported by the experimental data showing that disrupting actin by cytochalasin D abolished the flow effect on NHE3 activity and trafficking [17, 19]. However, if brush border microvilli have a flow mechanosensor function, the flow-induced changes in torque and in transport activity should be directly proportional to one another. By using the following simplified equation generated from Fig. 3a, established by our group, we were able to calculate and compare the changes in torque and the changes in fluid and HCO_3^- absorption at perfusion rates of 10, 15, 20, and 25 using the reference value at flow rate of 5 nl/min. The effects of Na^+ and HCO_3^- on changing flow rate on torque and changes of torque on proximal tubule transport were compared. As shown in Fig. 3b, the relationship between increasing perfusion rates and changes in torque (T), the relationship between J_v and T (Fig. 3c), and between J_{HCO_3} and T (Fig. 3d) are all almost perfectly linear, suggesting that the flow-modulated changes of tubule transport activity are directly modulated by the changes in microvillus torque. Hydrodynamic drag and torque on the microvilli can be increased by increasing the tubular flow rate or by increasing the fluid viscosity without changing the flow rate. Accordingly, whether Na^+ absorption is altered by increasing the viscosity of the tubular per-fusion solution was evaluated. As shown in Fig. 4a, increasing the viscosity by adding dextran-80 to the perfusing fluid did not change the maximal absorption rate, but the flow-dependent transport curve was shifted to the left, supporting the theory that flow-stimulated Na^+ transport is torque dependent. From the following equation (Eq. 1), to calculate torque, one sees that the flow rate (Q) and the viscosity (μ) are in direct proportion with microvillus T , while tubule cross section (R^2) is inversely proportional.

$$\frac{T}{T_r} = \frac{R^2}{R_r^2} \left(\frac{1 + \frac{L+\delta}{R} + \frac{L^2}{2R^2}}{1 + \frac{L+\delta}{R_r} + \frac{L^2}{2R_r^2}} \right) \left(\frac{\mu}{\mu_r} \right) \frac{Q}{Q_r} \quad (1)$$

r – index to reference value

R – inner tubule radius with brush border

L – length of the microvilli ($L = 2.5 \mu\text{m}$)

δ – microvillus tip interaction layer ($\delta = 150 \text{ nm}$)

μ – fluid viscosity

Q – flow rate in the tubule

If the flow-stimulated transport is torque dependent, then reduction of torque by increasing the tubule diameter would reduce flow-stimulated tubule transport. We then reconsidered the data of Burg and Orloff [10], widely cited as a negative effect of flow on proximal sodium reabsorption in isolated rabbit proximal tubules. As shown in Fig. 4b, inner diameters of the proximal tubule increased when flow was high in both rabbit and mouse tubules. However, rabbit proximal tubules appear more distensible than mouse. Tubule diameter increased 41% in rabbit tubule and 30% in mouse tubule when flow rate increased from 6 to 18 nl/min. Indeed, when flow rate was increased from 6 to 18 nl/min, torque and J_v increased by 38 and

37%, respectively, in rabbit proximal tubules. Similarly, torque and J_v increased by 61 and 60%, respectively, in mouse proximal tubules (Fig. 4c, d), indicating proportional changes in torque and J_v in response to flow exhibited in both species. As the change in torque is inversely related to the change in tubule diameter (Eq. 2), the increase in torque is significantly lower in rabbit than in mouse tubules due to the greater enlargement of the tubule lumen in the former. Thus, the reason for the absence of flow-stimulated Na^+ absorption in rabbit proximal tubules in vitro is indicated by our equation for torque-dependent transport [43].

$$T_{\text{tot}} = \frac{1}{N} \cdot \frac{8\mu}{R^4} \cdot Q \cdot L \left[R^2 + R(L + \delta) \frac{L^2}{2} \right] \quad (2)$$

A mathematical model of rat proximal tubule was extended to include calculation of microvillus torque using the equation above and to incorporate torque-dependent solute transport in a compliant tubule [50]. In the model calculations, torque-dependent scaling of luminal membrane transporter density (either as an ensemble or just NHE3 alone) had a relatively small impact on overall Na^+ reabsorption and could produce a lethal derangement of cell volume; coordinated regulation of luminal and peritubular transporters was required to represent the overall impact of luminal flow on Na^+ reabsorption. In all of our experimental studies of flow-dependent transport in mouse tubules, this was achieved with virtually no change in tubule cell volume. The model calculations suggest that this observation is strong evidence for proportional luminal and peritubular effects of flow on transporter density. The impact of microvillus torque on transporter density was captured with a single sensitivity parameter. When the magnitude of this sensitivity was taken slightly lower than suggested by our observations in mouse tubules, the rat model tubule showed perfusion-absorption balance in good agreement with observations in the rat, over a broader range of inlet flows. In simulation of hyperglycemia, torque-dependent transport attenuated the diuretic effect and brought the model tubule into closer agreement with experimental observation in the rat. With torque modulation, end-luminal volume flow was found to be nearly constant over the range of ambient glucose (20–80 mM), and end-luminal glucose concentration was found to approximate the perfusate concentration. Both of these predictions brought the model tubule into agreement with micropuncture observations of hyperglycemia in the rat [42].

Regulation of flow-activated proximal tubule function by second messengers

The three major signal transduction pathways that are known to regulate kidney electrolyte transport, Ang II, dopamine, and calcium signals, have been investigated by our group. We have characterized the functional roles of each of them in the modulation of GTB as described in the following.

Ang II

Ang II, via activation of its receptors, is an important regulator of proximal Na^+ and HCO_3^- transport in the kidney. In the intact animal, selective blockade of angiotensin II is diuretic, so that under normal conditions, it appears that there is tonic stimulation by angiotensin II [54]. In intact rat tubules, the most prominent effect of angiotensin II, in picomolar concentrations, is the stimulation of NaHCO_3 reabsorption via Na^+/H^+ exchange [27], and this is mediated by binding to AT1 receptors [53]. Ang II is produced locally and secreted into tubule fluid, and the AT1 receptor is expressed on both apical and basolateral membranes of the proximal tubule [8, 9]. Using both in vivo and in vitro microperfusion techniques, we have reported, along with other investigators, that luminal administration of Ang II produced biphasic effects on proximal tubule transport [27, 44]. Previous studies reported that Ang II plays a role in the regulation of GTB [56], but others have indicated that the AT1 receptor is not critical for GTB [12]. We examined the effect of the AT1 receptor blocker losartan, the knockout of AT1a, and the role of AT2 in flow-mediated PT transport. The AT1 blocker losartan reduced absolute Na^+ and HCO_3^- absorption at both low and high flow rates, but did not affect the flow-stimulated Na^+ and HCO_3^- transport [15]. In contrast, in addition to significant reductions on Na^+ and HCO_3^- absorption on both low and high flow rates, the flow-stimulated Na^+ absorption was completely abrogated and flow-stimulated HCO_3^- absorption was significantly reduced in AT1a KO mouse (Fig. 5). These differences between AT1 blockage and the knockout are possibly due to reduced NHE3 expression in the kidney of AT1a KO mice [15]. The altered physiology underlying this reduced NHE3 expression may also lead to a reduction in flow-increased Na^+ and HCO_3^- absorption in proximal tubules of AT1a KO mice. The remaining flow-stimulated HCO_3^- absorption is due to flow activation of H-ATPase activity [15]. As shown in Fig. 5, in WT mice, inhibition of the AT2 receptor by PD123319 increased both Na^+ and HCO_3^- absorption but did not affect the flow-induced changes on tubule transport. In addition, PD123319 had no effect on Na and HCO_3^- absorption in AT1a KO mice, indicating that AT2 receptor function is not regulated in AT1a KO mice. These results indicate that the AT1 receptor is important to maximize the NHE activity activated by flow. However, it is not critical for the flow-stimulated HCO_3^- transport, which still exists when the inhibitors are present or when the AT1a receptor is knocked out.

Dopamine

Dopamine is the most important natriuretic signal to the proximal tubule [2, 11]. Urinary dopamine derives largely from circulating L-DOPA [4, 6], and proximal tubules are a source for this excreted dopamine [5]. Within the proximal tubule, conversion from DOPA to dopamine is due to the enzyme L-amino acid decarboxylase (L-AADC), and the importance of the local generation of dopamine was underscored by the demonstration that maximal L-AADC activity is modulated by dietary salt intake [39]. Proximal tubules are also a target for the locally generated dopamine, with inhibition of apical NHE3 and basolateral Na/K-ATPase [2]. A mouse model in which the intrarenal dopamine is deficient becomes hypertensive with increased NHE3, NKCC2, NBC, NCC, and AQP2 mRNA expressions in the kidney [55], consistent with dopa-mine downregulation of Na^+ transport in the kidney. In OK cells, the dopamine-induced acute decrease in surface NHE3 is dependent on both dopamine (DA)1 and DA2 receptors, and dopamine-stimulated NHE3 endocytosis can be

blocked by protein kinase A (PKA) inhibition or by mutation of protein kinase A target serines on NHE3 [26]. At least five iso-forms of dopamine receptors (DA1 to DA5) have been identified, and they have all been found in the mammalian kidney [34]. The DA1 receptor is expressed in kidney proximal tubules at both the apical and basolateral sides, and the DA2 receptor is also detected in the proximal tubule and distal nephron segments [34]. Previous studies show that both DA1 and DA2 receptors regulate Na^+ transport in proximal tubule and proximal tubule cells [7, 26]. We examined whether the effect of dopamine on flow-stimulated Na^+ absorption is due to a DA1 and/or a DA2 receptor-mediated mechanism.

Luminal dopamine did not change J_v , J_{Na} , and J_{HCO_3} at low flow rates but completely abolished the increments of Na^+ absorption by flow (Fig. 6a) and partially inhibited the flow-stimulated HCO_3^- absorption (Fig. 6b). The remaining flow-stimulated HCO_3^- absorption was completely abolished by bafilomycin [18]. In addition, the DA1 receptor blocker, SCH23390, and the PKA inhibitor, H89, blocked the effect of exogenous dopamine and produced a two fold to three fold increase in the sensitivity of proximal Na^+ reabsorption to luminal flow rate [18]. (We defined “torque sensitivity” as the ratio of the fractional change in flux to the fractional change in microvillus torque.) A comparison of DA1 and DA2 receptor functions in torque-dependent changes in Na and HCO_3^- absorption displayed in Fig. 6 indicated that the DA1 receptor is the major receptor responding to dopamine effects on proximal tubule. These results are consistent with the hypothesis that increased flow causes redistribution of NHE3 within the luminal membrane to a domain where it is active; this activation is essential for flow-stimulated Na^+ and HCO_3^- absorption. It is also the first demonstration that tubule sensitivity to flow-induced changes in torque can be regulated.

Calcium signals and the IP3 receptor

Calcium signals are the major second messenger mechanisms for primary cilium-mediated mechanosensation in MDCK cells [36], in cortical collecting duct [52], and in kidney epithelial cells [35]. However, regulation of flow effect by calcium signals in the proximal tubule has never been studied. In the collecting duct, flow-stimulated K^+ secretion is dependent on apical calcium entry; removal of Ca^{2+} from the luminal perfusate prevents luminal Ca^{2+} entry and abrogates flow stimulation of net K^+ secretion [29]. It is not known whether the proximal tubule cell response of Ca^{2+} is similar to other cell types. In the proximal tubule, however, it is generally believed that an increase in cytosolic Ca^{2+} is associated with a decrease in NHE3-mediated Na^+ reabsorption [48]. It has been suggested that in the proximal tubule, cytosolic Ca^{2+} functions as a negative feedback signal at the luminal membrane to blunt Na^+ entry [21]. For those reasons, the effect of low and high luminal Ca^{2+} , the Ca^{2+} chelator BAPTA-AM, the IP3 receptor antagonist 2-APB, and the Ca-ATPase inhibitor thapsigargin on flow-stimulated Na^+ and HCO_3^- absorption were examined to investigate the role of calcium signals in flow-activated proximal tubule transport [16]. As shown in Fig. 7a, c, luminal high Ca^{2+} reduced torque-dependent Na^+ and HCO_3^- absorption but low Ca^{2+} had no effect, indicating that Ca^{2+} influx does not mediate flow-stimulated Na^+ and HCO_3^- absorption and increasing cytosolic Ca^{2+} downregulates proximal tubule transport. Additional salient findings of this work are clamping cytosolic

Ca^{2+} with a chelator (BAPTA) or application of the IP3 receptor antagonist 2-APB abrogates the flow-dependent increase of Na^+ and HCO_3^- absorption (Fig. 7b, d). These observations suggest that a local Ca^{2+} puff, triggered by IP3, is part of the signal transduction pathway from the force on the actin cytoskeleton to the activation of NHE3. This activation could plausibly be insertion of new transporters from vesicle reservoirs or shuttling of NHE3 already within the luminal membrane from a basal location to a more available site. Such a role for the IP3 receptor is consistent with its function in other cells (e.g., endocrine secretion, lymphocyte function, or synaptic transmission) but has not been previously identified in kidney proximal tubule [20].

Summary and Conclusion

Recent work has made major progress in defining the underlying cellular mechanisms of flow-regulated proximal tubule transport. First, it has been demonstrated that perfusion-absorption balance is present in the isolated perfused proximal tubule of the mouse kidney, and it is independent of neuronal control and systemic hormonal regulation [14]. Higher axial flow rates stimulate sodium and bicarbonate absorption by increased apical membrane Na^+/H^+ transporter and H-ATPase activities [17]. It is also evident that fluid shear stress stimulates NHE3 exocytosis and trafficking to the apical membrane of the proximal tubule cells [19]. Second, experimental data and modeling calculations provide strong evidence that brush border microvilli function as flow sensors in the proximal tubule [14]. Flow-induced changes of proximal tubule absorption depend on the changes of torque (bending moment) on the microvilli (as shown in Fig. 8), and an intact actin cytoskeleton is required to transduce signals from the brush border to cell and alter transport activity, NHE3 expression, and trafficking [19]. A simplified theoretical formula was established for calculating the forces and torques on the micro-villi, which includes the effect of changing tubular diameter [14]. Changes in luminal diameter are inversely correlated to the changes in torque, and the greater compliance of the rabbit tubule compared to the mouse tubule resolves a four-decade-old mystery as to why the flow dependence of transport found in vivo did not appear to be present in vitro [43, 47]. Third, dopamine decreases tubule sensitivity in microvillus torque-dependent Na transport and DA1 antagonist increased the transport sensitivity to torque [18]. These results are consistent with the hypothesis that increased flow causes redistribution of NHE3 within the apical membrane, as found in proximal tubule cells [18]; this activation is essential for flow-stimulated Na^+ and HCO_3^- absorption. Finally, we have demonstrated that IP3 receptor-mediated intracellular Ca^{2+} signaling plays a critical role in transduction of microvillus torque to increase Na^+ and HCO_3^- absorption [16]. High luminal Ca^{2+} downregulates Na^+ and HCO_3^- absorption in the proximal tubule, and increasing Ca^{2+} influx has no impact on flow-stimulated proximal tubule transport [16]. These results indicate that the role of Ca^{2+} signals in flow regulation of Na^+ and HCO_3^- absorption in proximal tubule is different from its role in flow regulation of K secretion in the collecting duct [52]. Substantial uncertainties involving flow-dependent transport remain. Among all of the transporters within luminal and peritubular cell membranes, which are flow modulated? What is the signal cascade from microvillus to luminal transporter and the transduction pathway to peritubular transporters? What role does impaired GTB play in disorders of kidney function and in the progression of chronic kidney disease? It is known that fluid

shear stresses contribute to normal morpho-genesis and disease under pathological conditions in the cardiovascular system [3, 25, 31, 40, 41]. Thus, it will be important to define the roles of GTB under pathophysiological conditions in the kidney.

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Fig. 1.

Flow-activated proximal tubule Na^+ and HCO_3^- absorption. **a** Relationship between flow rate and fluid absorption (J_v , as an indicator for net Na^+ absorption in the proximal tubule). **b** Relationship between flow rate and HCO_3^- absorption (J_{HCO_3}) in proximal tubules. J_v and J_{HCO_3} were measured under perfusion rates of 5, 10, 15, 20, and 25 nl/min. * $P < 0.05$ compared with the low flow rate (5 nl/min). **c, d** Effects of EIPA and bafilomycin on J_v (**c**) and J_{HCO_3} (**d**) in proximal tubules. J_v and J_{HCO_3} were measured at low (5 nl/min) and high (20 nl/min) perfusion rates in the absence and presence of 10 M EIPA, 0.1 M bafilomycin, and EIPA plus bafilomycin, respectively, in the luminal perfusion solution. *Bar graph* shows the delta changes of J_v or J_{HCO_3} from low flow rate. *Asterisk* indicates the significant difference from control ($P < 0.05$). (Reproduced with permission from refs. [14, 17])

Fig. 2.

Effect of FSS on NHE3, Na/K-ATPase, and V-ATPase trafficking and expression in confluent MPT cells. Immunofluorescence (IF) images of MPT cells under control (CTL) (a), FSS (b), and cytochalasin D (CDFSS) conditions (c). The antibody stained for NHE3, Na/K-ATPase, and H-ATPase (red) and actin filaments (green), respectively. **d** Distributions of normalized NHE3-, Na/K-ATPase-, or V-ATPase-stained fluorescence intensity. The values were normalized such that the mean maximum value in the stimulation group was 100% in each experiment. **e** Western blot analysis showing NHE3, Na/K-ATPase, or V-ATPase levels under control and FSS conditions. Expression levels of ion transporters were normalized on actin expression in the same lane. Results in bar graph are means $\pm$ SE and are percentage of control condition (* P < 0.05). (Reproduced with permission from ref. [19]). **a** Effect of FSS on NHE3 trafficking and expression in MPT cells. In control, NHE3 scattered in the cytoplasm and mainly localized below the actin rings (arrows). After 3-h

FSS at 0.2 dynes/cm², the NHE3 staining was stronger and the stained areas were larger than those in control cells. The majority of NHE3 now localize at the apical region. After FSS, the NHE3 staining was stronger and the stained areas were larger than those in control cells. The majority of NHE3 now localize at the apical region. **b** Effect of FSS on Na/KATPase trafficking and expression in MPT cells. Na/K-ATPase weakly distributed mainly at the basolateral membrane with some intensive staining near the cell-cell contacts (*arrows*). After FSS, an elevation in membrane Na/K-ATPase expression was observed mainly at the cell periphery (*arrows*). Colocalization of Na/K-ATPase and actin was prominent (*X-Z* image, *inset*). CD significantly blunted the flow-dependent effects (*green line*). **c** Effect of FSS on V-ATPase trafficking and expression in MPT cells. V-ATPase was expressed more evenly and brightly in every cell. There is a translocation of V-ATPase witnessed in the *X-Z* image. CD did not affect the flow-dependent upregulation and translocation of V-ATPase. **d** The fluorescence intensity of the cells was shifted to the right (cell apex) after application of FSS (*red*). This effect was blunted with the addition of CD (green)

Analysis of the changes of transport activity and total torque in response to increased perfusion rates. **a** Simplified proximal tubule model for calculating microvillus torque. Detailed information can be found in ref. [14]. **b** The relationship between increasing perfusion rates and changes in torque. T_{total} total torque of the microvilli (body and tip); T_r reference value of the total torque at the perfusion rate of 5 nl/min; T/T_r ratio of total torque at related perfusion rates of 10, 15, 20, and 25 nl/min to the reference value at a perfusion rate of 5 nl/min. **c** The proximal-tubule transport Na^+ ($J_v/J_{v,r}$) activity corresponding to changes of torque. **d** The proximal-tubule transport HCO_3^- ($J_{\text{HCO}_3}/J_{\text{HCO}_3,r}$) activity corresponding to changes of torque. The relationship between the changes of torque and changes of transport activity are proportional. (Reproduced with permission from refs. [14, 17])

Fig. 4.

Torque-dependent proximal tubule transport. **a** Effect of dextran-80 on flow-induced change on fluid absorption (J_v). **b** Flow-induced changes in inner tubular diameter (ID) in rabbit and mouse proximal tubule. **c** Comparison of fluid absorption (J_v) under low and high tubule flow rates between rabbit and mouse tubules. (Reproduced with permission from refs. [14, 43])

Fig. 5.

Role of AT1a and AT2 receptors in flow-mediated sodium (a) and bicarbonate (b) absorption (J_{Na} and J_{HCO_3}) in proximal tubules of mouse kidney. J_{Na} and J_{HCO_3} were measured at low and high perfusion rates in absence and presence of AT2 inhibitor, PD123319 (10^{-6} M) in WT and AT1a KO mice. * $P < 0.05$, compared to low flow rates in the same group; † $P < 0.05$, compared to the control at the similar flow rate. (Reproduced with permission from ref. [15])

Fig. 6.

Role of dopamine and dopamine receptor (DA1) and DA2 in flow-induced changes in torque (T) and sodium (A), bicarbonate (B) reabsorption in proximal tubules. Dopamine (10^{-5} M), SCH23390 (DA1 antagonist, 10^{-5} M), and sulpiride (DA2 antagonist, 10^{-5} M) were added to the luminal perfusate. * $P < 0.05$ compared from the control group. (Reproduced with permission from ref. [18])

Fig. 7.

Effect of low or high Ca^{2+} and Ca^{2+} inhibitors on flow-induced changes in torque-dependent Na^+ and HCO_3^- absorption. Data are represented as the ratio of changes in ion absorption and changes in torque by flow a Na^+ absorption ($J_{Na}/J_{Na,r})/(\Delta T/T_r)$ and b HCO_3^- absorption ($J_{HCO_3}/J_{HCO_3,r})/(\Delta T/T_r)$. r reference volume from low flow rate (5 nl/min). * $P < 0.05$, compared with the change in control group. The cell-permeable calcium chelator BAPTA-AM (2×10^{-5} M) and inositol 1,4,5-trisphosphate (IP3) receptor antagonist 2-aminoethoxydiphenyl borate (2-APB; 10^{-5} M) were added to the luminal perfusate, respectively. Asterisk indicates the significant difference from the control group ($P < 0.05$). (Reproduced with permission from ref. [16])

Fig. 8.

Schematic model of flow-activated luminal and peritubular membrane transporters of proximal tubule. **a** Brush border microvillus torque and membrane transporters operating under low axial flow rate. **b** High axial flow rate increases microvillus torque (bending moment) and increases NHE3 and H^+ -ATPase activity and expression. We can find doubling of transepithelial Na^+ fluxes (Fig. 3) with no change in epithelial cell height (i.e., cell volume). This must imply activation of peritubular membrane transporters, but which transporters and their activating signals are unknown. In our studies of PT cells in culture, we were able to demonstrate traffic of Na,K-ATPase with changes in apical shear stress [19]. The mechanism of cross talks between apical and basolateral membrane has not been identified