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■ REVIEW ■

Vesicular Transport as a New Paradigm in Short-Term Regulation of Transepithelial Transport

The vectorial transepithelial transport of water and electrolytes in the renal epithelium is achieved by the polarized distribution of various transport proteins in the apical and basolateral membrane. The short-term regulation of transepithelial transport has been traditionally thought to be mediated by kinetic alterations of transporter without changing the number of transporters. However, a growing body of recent evidence supports the possibility that the stimulus-dependent recycling of transporter-carrying vesicles can alter the abundance of transporters in the plasma membrane in parallel changes in transepithelial transport functions. The abundance of transporters in the plasma membrane is determined by net balance between stimulus-dependent exocytic insertion of transporters into and endocytic retrieval of them from the plasma membrane. The vesicular recycling occurs along the tracts of the actin microfilaments and microtubules with associated motors. This review is to highlight the importance of vesicular transport in the short-term regulatory process of transepithelial transport in the renal epithelium. In the short-term regulation of many other renal transporters, vesicular transport is likely to be also involved. Thus, vesicular transport is now emerged as a wide-spread general regulatory mechanism involved in short-term regulation of renal functions.

Key Words: *Vesicle Recycling; Exocytosis; Endocytosis; H(+)-Transporting ATP Synthase; Chemiosmosis; Proton-Motive Force; Actins, Microfilaments; Microtubules; Epithelial Transport*

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INTRODUCTION

To maintain the constancy of the milieu interieur, the transepithelial transport functions of the kidney have to be continuously regulated to the changing external conditions. The vectorial transepithelial transport of water and electrolytes is achieved by distinct asymmetric distribution of a host of specialized transport proteins i.e., transporters such as channels, exchangers, co-transporters or pumps, as well as enzymes, hormonal receptors and cytoskeletal proteins in the apical and basolateral membrane domains of the renal epithelium (1). The rapid short-term changes in the order of a few minutes in the transepithelial transport of water and electrolytes in the kidney have been generally thought to be effected by kinetic modulations of transport proteins with respect to substrate concentrations, affinities for substrates, turnover rates or activation of quiescent proteins already resident in the membranes by phosphorylation/dephosphorylation (Fig. 1). A classical example is the rapid stimulation of Na^+ transport in the tight urinary epithelia by vasopressin. This hormone increases Na^+ influx across the apical membrane into epithelial cells with a subsequent increased Na^+ efflux out of cells across the basolateral membrane by Na^+ pumps, which is activated secondarily to an increased substrate concentration of intracellular Na^+ (2). In this classical regulatory paradigm, there is no changes in the number or abundance of transport proteins in the plasma membrane. Much of recently emerging evidence, however, reveals a new paradigm of regulatory mechanism of transepithelial transport in the kidney. That is, rapid changes in the transepithelial transport involve changes in the number of transport proteins in the plasma membrane through an exocytic insertion of transporter-containing vesicles into the plasma membrane and their endocytic retrieval. In this new paradigm of vesicle mediated short-term regulation of transepithelial transport, the abundance of transport proteins increase or decrease, that is determined by the rate of exocytosis and endocytosis. The short-term regulatory mechanism by vesicular transport described in this review differs from the long-term regulatory mechanisms requiring de

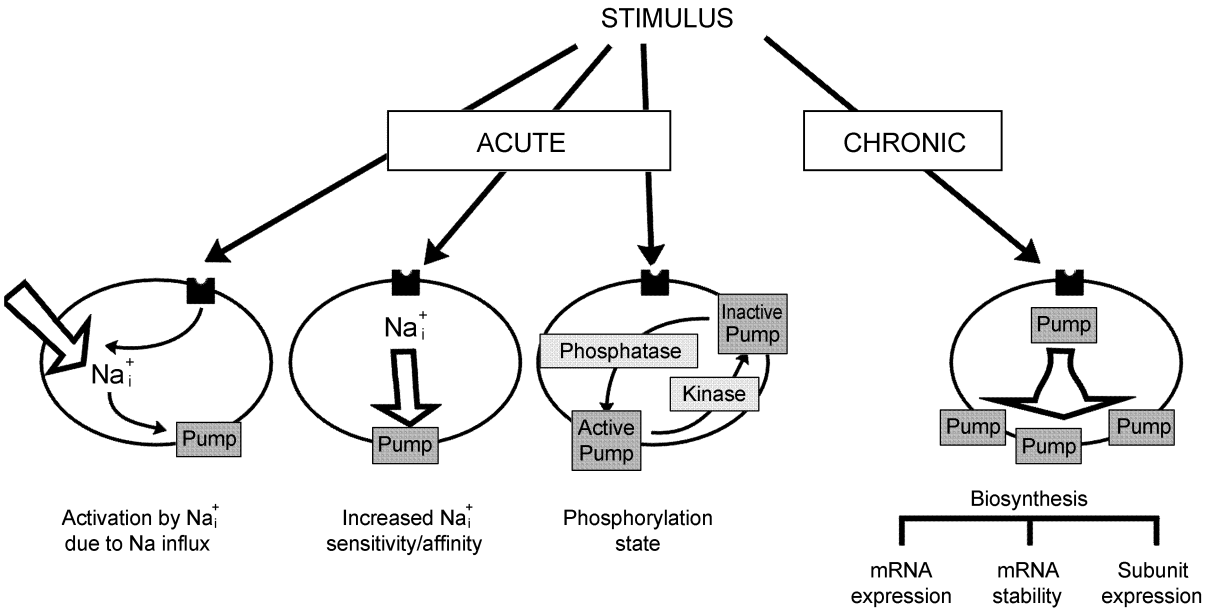

Fig. 1. Traditional concept of short (acute)- and long (chronic)-term regulation.

novo synthesis of new transporters which occur over a period of hours or even days. Five criteria for the short-term regulation of transepithelial transport mediated by vesicular transports include: 1) existence of transport proteins in both plasma membrane and intracellular vesicle membrane, 2) a reciprocal change in the abundance of transport protein between plasma membrane and intracellular vesicle membrane, 3) short span of initiation of response within a few minute with full response in an hour, 4) dependence of intact cytoskeleton of actin microfilaments and microtubules, 5) independence of de novo synthesis of transport protein, i.e., insensitive to transcriptional inhibitor actinomyosin D and translational inhibitor cycloheximide.

A brief historical note seems to provide an underlying basis for the development of current ideas on the vesicular transport as an important short-term regulatory mechanism of transepithelial transport. In 1952, the first vesicular transport was invoked in the quantum release of neurotransmitters at the neuromuscular junction (3). This was followed by secretion of hormones from endocrine cells via exocytic release through fusion of secretory vesicles with plasma membrane (4). A similar vesicular transport was found in the stimulation of H^+ secretion from the gastric oxyntic cells by histamine (5). Not only release but also the cellular uptake of LDL was also recognized as vesicular transport, termed a receptor-mediated endocytosis (7). In the renal epithelium, the first vesicular transport has been invoked in H_2O transport in response to vasopressin (8) followed by H^+ transport to CO_2 (9), and Na^+ transport to hydrostatic pressure (10).

INTRACELLULAR VESICULAR TRAFFICKING

The intracellular milieu is highly compartmentalized by endomembrane which constitute the exocytic and endocytic organelles and vesicles. Proteins and lipids in the plasma membrane are continually but slowly being replaced by the exocytic insertion of newly synthesized proteins and lipids and the endocytic retrieval of them from the plasma membrane via vesicular transports, even in the absence of any apparent stimulus. This sort of recycling of membrane proteins and lipids is referred to as constitutive vesicular transport. Thus the abundance of transport proteins and lipids in the plasma membrane at a given time is net balance between the rate of exocytic insertion and endocytic retrieval. In the presence of extracellular stimulus, the vesicular transport involved in the regulation of transepithelial transport alters the abundance of transport proteins by either increasing exocytic insertion into or endocytic retrieval from the plasma membrane (Fig. 2). Upon the removal of stimulus, the altered abundance of membrane proteins returns back to the level prior to the stimulus. Thus, transport proteins undergo recycling between the plasma membrane and the intracellular endosomes (or endosome like vesicles) whose endomembranes contain recycling transport proteins.

Most vesicular transport, recycling between plasma membrane and intracellular compartments, occurs along the actin microfilament and microtubule track and is driven by their associated motor proteins (1, 11). The actin microfilament is assembled by polymerization of globular (G) actin with a number of diverse actin-binding

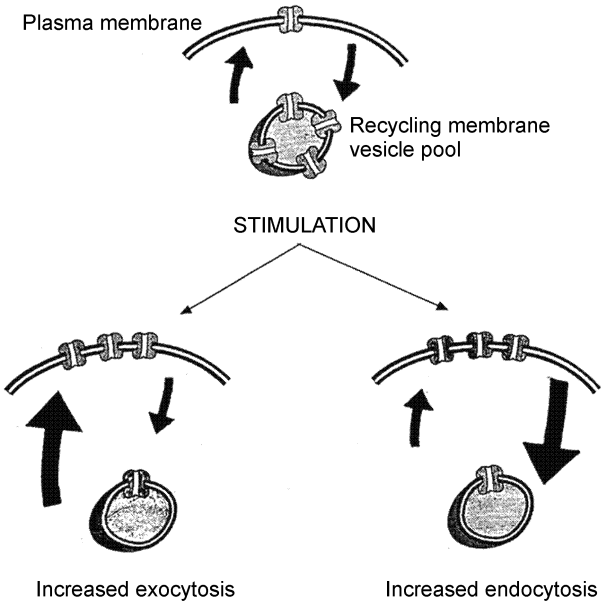

Fig. 2. Regulation of abundance of transporter in the plasma membrane by exocytic insertion (left) and endocytic retrieval (right).

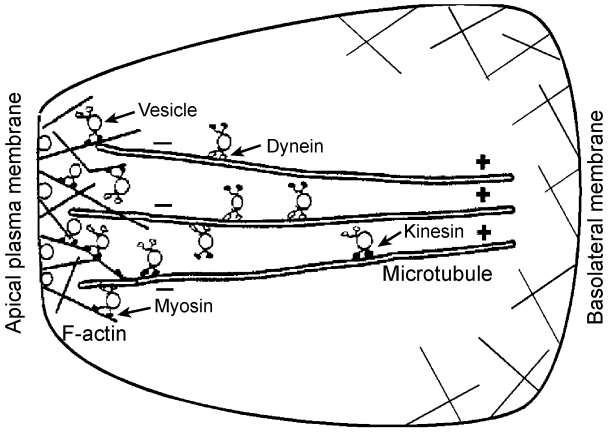

Fig. 3. Vesicular transport along the microtubular track from subapical domain to cell center motored by kinesin and in reverse direction by dynein. The transport along the actin microfilament track from subapical domain to apical membrane motored by myosin.

proteins and actin-related proteins (Arp). The actin microfilament is highly dynamic and polarized in that globular actin is continuously assembled at the barbed (growing) end and disassembled at the other pointed end. In the renal epithelia, actin microfilaments are predominantly localized in the subapical domains, forming latticelike subapical actin network (Fig. 3). The subapical actin network has been conceived as a physical barrier preventing recycling vesicle from free access to the apical membrane and as a physical structure to anchor vesicles in the vicinity of the apical membrane.

The actin microfilament is believed to provide a track for myosin-driven vesicular transport. There are now about 14 distinct classes of myosins among which myosin I and V classes are likely to play a role in vesicular transport along the microfilament track (11-13). Current evidence suggests that the short-range movements of recycling vesicles between the apical membrane and its proximity subapical domain is myosin-driven along the actin microfilament track whereas the long-range movement between the cell center to subapical domain through cytoplasm is driven by either kinesine or cytoplasmic dynein along the microtubule track (Fig. 3) (14).

The molecular mechanisms whereby myosin-driven vesicle movement along the actin microfilament-tracks are regulated in response to extracellular stimuli are largely unknown. Recent evidence suggests that members of Rho GTPase family (Rho, Rac, Cdc42 etc.) have been identified as key players in controlling organization and functions of microfilaments (15).

Activation of GTP-bound Rho leads to inhibition of myosin light chain (MLC) phosphatase via phosphorylation of myosin-binding subunit (MBS), resulting in an assembly of microfilaments and activation of type II myosin ATPase in nonmuscle cells (15). The Rac/Cdc42 is known to recruit WASP (Wiskott-Aldrich syndrome protein) on the vesicle surface and activates actin filament nucleating activity of Arp2/3 complex. WASP binds Src homology 3 (SH3) at the tail domain of myosin I. Thus an activation of Rac/Cdc42 can induce an association between vesicles and its translocating motors concomitant with an increased motor activity along microfilament tracks (16). A marked inhibition of vesicular movements during mitosis was correlated with phosphorylation of myosin V heavy chain with dissociation of myosin from vesicles (17). A calcium-induced disassociation of myosin V from synaptic vesicles was also reported (12). Unlike myosin V, phosphorylation of myosin I heavy chain activates actin-based motility and myosin ATPase activity (18). In summary, the myosin-driven vesicle movements along the actin microfilament track can be modulated in a variety of modes in response to stimuli through activation/inactivation of motor activity, assembly/disassembly of actin filaments and association/dissociation of myosin motor with its cargo vesicles. But much remains obscure.

The microtubules are polymers of α-and β-tubulin dimers and their polymerization continuously occurs at one end termed plus (+) end with concomitant depolymerization occurring at the other minus (−) end. This polarity determines the directionality of vesicle movements such that the kinesin motors move to the plus end whereas the cytoplasmic dynein motors move to the minus end (1, 19). The microtubule organization in the renal epithelia is unique in that its minus end is posi-

tioned at the apical membrane with the plus end extending across cytoplasm toward the basolateral membrane (19). In most nonpolarized cells the minus end of the microtubule is connected with perinuclear centrosome with the plus end radiating toward the cell periphery. It has been generally thought that the long-range bidirectional vesicle movements between cell center and subapical actin network are carried by the microtubule system (1, 14).

The molecular mechanisms for the control of the microtubule-dependent vesicle movements are complex and under active investigation (20). In much the same manner as the actin-myosin system, the microtubule-dependent vesicle movements are known to be modulated by altering motor activity, assembly/disassembly of microtubules and association/dissociation of motors with their cargo vesicles through reversible phosphorylation/dephosphorylation cascades (11, 21-23).

One of the hallmarks for the vesicle-mediated transport has been sensitivity to destabilizing agents of the actin microfilaments (cytochalasins) or microtubules (colchicine) or conversely to stabilizing agents (phalloidin, taxol). When interpreting results from such experiments with use of those agents, cautions must be always kept in mind regarding their limited specificity and dynamic nature of the cytoskeleton. The problem is complicated even more by several lines of recent evidence suggesting that vesicle movements along the actin microfilament and microtubule tracks might not be independent but linked in a coordinated manner (13, 22). It is therefore possible that effects of microtubule destabilizing/stabilizing agents might not be through their actions exclusively on the microtubule-dependent vesicle transport but also on the actin microfilament-dependent vesicle transport.

RENAL TRANSPORT PROTEINS REGULATED BY VESICULAR TRANSPORT

Vasopressin-regulated aquaporin 2 (AQP2)

The first and best studied example of vesicular transport involved in the regulation of renal epithelial functions is the vasopressin-induced water permeability of the collecting duct. Twenty years ago, the vasopressin-induced particle aggregates, speculated then water channel protein, were observed in the apical membrane of toad urinary bladder, a model of mammalian collecting duct (8). The gene of collecting-duct water channel was cloned, and the channel protein, now designated as aquaporin 2 (AQP2), was characterized as an integral membrane glycoprotein of 35-50 kDa with six transmembrane domains. By immunoelectron microscopy and immunolocalization of AQP2, the channel proteins located in the subapical endosome of principal cells of collecting duct. These AQP2 were translocated to the apical membrane within 1 min after stimulation with vasopressin. Upon removal of vasopressin, the inserted channels were retrieved in the first 3-5 min by an accelerated endocytosis and returned back to the subapical endosomes (24-25). The insertion of AQP2 into and its retrieval from the apical membrane were functionally paralleled with an increase in the osmotic water permeability and its reversal. There recent findings in the collecting duct principal cells of the mammalian kidney are consistent with membrane shuttle hypothesis postulated 20 years ago on the basis of experiments with toad urinary bladder (8). It is now well established that an increased exocytic insertion of AQP2 into the apical membrane of the principal cell in response to vasopressin is functionally coupled with an increased water permeability (24-26).

Several important questions still remain, however, especially regarding the underlying molecular mechanisms by which vasopressin translocates AQP2 carrying vesicle from the intracellular pool exclusively to the apical membrane, finally leading to fusion with and subsequent insertion of AQP2 into the apical membrane of the principal cell. The binding of vasopressin to its V_2 receptors on the basolateral membrane activates adenylate cyclase and generates cAMP. cAMP activates protein kinase A (PKA) that phosphorylates its target proteins. The activated PKA is shown to phosphorylate serine-256 residue of the AQP C-terminus in the vesicle membrane (24-26). This phosphorylation is one known tragetting signal leading to the translocation of AQP2 exclusively to the apical membrane. In LLC-PK1 cells expressing AQP2, vasopressin was reported to translocate the water channel to the basolateral membrane rather than to the apical membrane, suggesting that AQP2 phosphorylation alone may not be sufficient for the apical membrane targeting. In view of the reports that various agents disrupting microtubules and actin microfilaments interfere with the effect of vasopressin on H_2O transport in renal epithelia, vasopressin-induced PKA-dependent phosphorylation of the cytoskeleton, in addition to AQP2, is likely to involve in the translocation of AQP2-containing vesicles. In nonmuscle cells PKA-dependent phosphorylation of myosin light chain kinase (MLCK) is known to disassemble and reorganize actin microfilaments (27). One school of thought speculated that vasopressin, via the cAMP-PKA activation signalling pathway, might reorganize subapical membrane actin network and remove a physical barrier, allowing the AQP2-carrying vesicles free access to the apical membrane (26). But cytochalasin D was found to disrupt actin microfilaments and also interfered the effect of vasopressin (25), making the above hypothesis ques-

tionable.

The localization of AQP2 and other transport proteins to the apical membrane is also microtubule-dependent (25, 26). Disruption of microtubules with colchicine or nocodazole redistributes AQP2 (as well as several apical membrane resident proteins into cytoplasmic vesicles with little localization in the apical membrane). The underlying molecular mechanism whereby the vasopressin elicited cAMP-PKA signalling cascade translocates the AQP2-carrying vesicle to the apical membrane along the microtubules with the minus end directed dynein motors remains obscure. In a pigment vesicle movements in the melanophore, a traditional model of microtubule-dependent vesicle movement, an activation of the cAMP-PKA signalling pathway is known to translocate the vesicle to the cell periphery using kinesin motors whereas an inactivation them translocates it the cell center (13, 23). A similar mechanism may be undergoing in the AQP2 translocation, but needs further study.

The final step of the vasopressin induced fusion of AQP2 carrying vesicle membrane with and consequential insertion of AQP2 into the apical membrane is an area of active investigation. Recent studies have focussed to identify the AQP2 vesicle- and apical- membrane proteins similar to those involved in the exocytic fusion of synaptic vesicles with presynaptic membranes in neurons (28). The vesicle associated protein (VAMP) in the vesicle membrane and syntaxin in the apical membrane of collecting duct principal cells have been identified (26). However, evidence from recent genetic studies on the synaptic transmission is not entirely consistent with the SNARE [soluble N-ethylmaleimide sensitive fusion protein (NSF) attached receptors] hypothesis postulated in neurons (29). Thus, the molecular mechanism for the exocytic insertion of AQP2 into the apical membrane of principal cell of the collecting duct is under intensive investigation.

Vacuolar H^+-ATPase

The regulation H^+ secretion by the kidney is long been known to be mediated by exocytic insertion of the H^+-ATPase into and its endocytic retrieval from the apical membrane of the renal tubule, in particular α-intercalated cells of the collecting duct (30, 31). The vacuolar type of H^+-ATPase is an electrogenic H^+-pump composed of 13 subunits (32). The vacuolar H^+-ATPase locates in the apical membrane of the collecting duct as well as in the subapical endosomes and lysosomes (30, 31). In the collecting duct α-intercalated cell, stimulation of H^+ secretion in response to an increased CO_2 tension or cytosolic acidification, for example, is associated with an increased apical membrane surface area as a result of an exocytic insertion of vacuolar H^+-ATPase located in the subapical endosomal membrane (30, 31). Disruption of microtubules with colchicine interferes with the translocation of the vacuolar H^+-ATPase to the apical membrane in response to CO_2 and spreads the vacuolar H^+-ATPase carrying endosomes throughout the cytoplasm of collecting duct intercalated cells (30, 31). Thus existing evidence is consistent with the vesicular hypothesis of regulation of H^+ secretion by exocytosis and endocytosis of the vacuolar H^+-ATPase, thus altering the number of the pump in the apical membrane. However, the molecular mechanisms with respect to intracellular signalling and its mode of action on the translocation of the vacuolar H^+-ATPase remain unknown.

As described below about reviewer's chemiosmotic hypothesis (33, 34), CO_2 via generating H^+ in the cytosol, may activate the vacuolar H^+-ATPase in the subapical endosomes and acidify the endosomal lumen leading to accumulation of HCl. An increased luminal HCl concentration with obligated osmotic H_2O influx would result in swelling of subapical endosome and subsequent fusion with juxtaposed apical membrane. In fact, the vasopressin-independent aquaporin 6 (AQP6) has been recently identified in intracellular vesicle membrane of rat renal cortex and medulla (35) through which H_2O can move in and out to reach rapid osmotic equilibrium of vesicle lumen with the cytoplasm.

Na^+/H^+ exchanger (NHE)

The Na^+/H^+ exchanger (NHE) is a phosphoglycoprotein of ~110 kDa in molecular weight with 10-12 transmembrane domains (36, 37). The NHE-1 plays an important role in transepithelial reabsorption of Na^+ and H^+ secretion in the kidney. The type 1 isoform of NHE (NHE-1) localizes almost exclusively to the basolateral membrane. The type 3 isoform (NHE-3) is present in the apical membrane of the proximal tubule and thick ascending Henle's loop (37). Unlike the NHE-1, the NHE-3 is present not only in the apical membrane but also in intracellular vesicles with cellubrevin, a marker of recycling endosomes (37). Parathyroid hormone (PTH) was found to rapidly (<2 min) decrease the NHE-3 activity of the apical membrane of the proximal renal tubule. The decreased NHE-3 activity and thus the number of NHE-3 was found to be mediated by endocytic retrieval of the exchanger resident in the apical membrane (38). Conversely, angiotensin II (AII) stimulates NHE-3 activity within 15 min by exocytic insertion of the exchanger into the apical membrane (39). Such activation and inhibition is attributable to a change in the maximal activity (V_{max}) of the exchanger without changing substrate affinity (K_m) (38, 39).

Na^+-P_i cotransporter

The regulation of inorganic phosphate (P_i) reabsorption in the proximal renal tubule is mediated by vesicular transport (40). The transport of P_i across the apical brush border membrane (BBM) by the type II sodium coupled P_i cotransporter (Npt2) is the rate-limiting step and the major step of regulation of P_i reabsorption (40). The Npt2 is about 80-90 kDa in glycosylated molecular weight with eight predicted transmembrane spanning domains (40). Parathyroid hormone (PTH) inhibits within delay of a few minutes. The decrease in P_i reabsorption is paralleled with a decrease in the abundance of Npt2 cotransporter in the apical BBM with a reciprocal increase in the subapical endosome membrane (41). The retreival of the Npt2 from the apical BBM is believed to be largely mediated by clathrin-dependent endocytosis (40, 41).

An increase or decrease in P_i transport across the apical BBM in response to a decrease or increase in dietary P_i intake, respectively occurs within 2 hr (42). Such alterations in P_i transport are closely associated with parallel changes in the abundance of Npt2 transporter in the apical BBM (42). The characteristic responses of P_i transport common to both PTH and dietary P_i intake are associated with changes in the transport maxima without changing affinity (Km). Changes in the abundance of Npt2 protein in the apical BBM were concomitant with reciprocal changes in its abundance in the subapical endosome, without changes in the total abundance of Npt2 protein nor its de novo synthesis (40, 41, 43).

The recycling of Npt2 transporter between the apical BBM and subapical endosomes is known to be microtubule-dependent (42, 43). PTH induces an endocytic retrieval of Npt2 from the apical BBM largely via cAMP-PKA and in part via PKC signalling cascade, but the underlying mechanism by which kinases trigger endocytosis of Npt2 remains unknown (44).

Epithelial Na^+ channel

The epithelial Na^+ channel (ENaC) in the apical membrane of the collecting duct principal cells is a heteroligomer of 3 homologous α-, β- and γ-subunit of 85-90 kDa each in molecular weight. Each subunit has two transmembrane domains, making the functioning ENaC with 6 transmembrane domains in the apical membrane.

The abundance of ENaC protein, i.e., apical Na^+ permeability, is modulated by cytoskeletal integrity (45) as well as by endocytosis (46), implicating an involvement of vesicular transport of ENaC in the regulation of the number of ENaC in the apical membrane. Vasopressin is a representative hormone to increase Na^+ transport in the tight epithelia within 5 min by an increase in the ENaC abundance via rapid translocation of ENaC from a subapical intracellular endosomes to the apical membrane (47, 48). The V_2 receptor of vasopressin mediates the activation of adenylate cyclase, the production of cAMP, and the subsequent activation of PKA. The phosphorylation of target protein(s) associated with ENaC translocation has not been identified yet. The increase in Na^+ permeability by vasopressin was found to associate with transient depolymerization of subapical actin network, raising possibility of removal of subapical physical barrier thereby allowing free access of ENaC to the apical membrane (49). Contrary to the prediction, however, depolymerization of actin network with cytochalasin D was found to inhibit vasopressin-induced ENaC translocation (49).

PKA is known to phosphorylate the β- and γ-subunits, but not α-subunit, of ENaC in their C-termini on serine and threonine residues (50). As for the AQP2, an alternative possibility is that the phosphorylation of the C-terminus on serine residue could be the targeting signal for the exocytic insertion of ENaC to the apical membrane. The endocytic retrieval of ENaC from the apical membrane is clathrin-mediated (46). The phosphorylation of the C-terminus of the β- and γ-subunits, but not α-subunit, on the tyrosine residue is the signal for the endocytosis (46). Thus, phosphorylation of different amino acid residues on the C-terminus could trigger exocytosis and endocytosis of ENaC, altering its abundance in the apical membrane.

Aldosterone is another principal hormone regulating Na^+ reabsorption in the collecting principal cells. Aldosterone has dual effects: one on the ENaC and the other on Na^+-pump (51). The effects show a typical time course of an early response (<3 hr) and a late response (>3 hr lasting 12-24 hr). The early response of increasing ENaC activity either mediated by an increased number of ENaC via de novo synthesis of ENaC or an activation of ENaC already resident in the apical membrane remains controversial. However, a recent study has shown a redistribution of ENaC α-subunits to the apical membrane from the diffusely and punctuate cytoplasmic pool (52). The early effect of aldosterone by vesicular transport of ENaC is also consistent with another recent report demonstrating that the abundance of α, β and γ subunit mRNA did not change during the first 3 hr of stimulation with aldosterone (53). With respect to nongenomic rapid effects, aldosterone was found to express serum and glucocorticoid-regulated kinase (sgk) in collecting ductal cells (54). The expression of this kinase was very rapid (<15 min) and insensitive to cycloheximide, an inhibitor of protein synthesis (54). As mentioned

above, the C-terminus of the β- and γ-subunit of ENaC was reported to be phosphorylated not only by vasopressin but also by aldosterone and insulin (50). Most importantly, the ENaC activity was far greater when ENaC and sgk were coexpressed than ENaC alone (54). These recent findings collectively support the possibility that aldosterone increases the abundance of ENaC in the apical membrane by phosphorylation of ENaC via expression of sgk, ultimately leading to exocytic insertion of ENaC into the apical membrane of the principal cells of the cortical collecting duct. In 1983 we postulated that aldosterone does not synthesize new Na^+ channel but activates previously existing non-functional channel (55); the non-functional channels we postulated then now appears to be resident in the cytoplasmic pool.

Na^+-pump (Na^+-K^+-ATPase)

The rapid activation of the Na^+-pump to an increased intracellular Na^+ concentration has been traditionally thought to occur by providing substrate to the pump. Surprisingly however, such intracellular Na^+ concentration dependent activation of the Na^+-pump in the collecting duct was aldosterone-dependent (56). Further study revealed that aldosterone elicited full activation of Na^+-pump within 1-2 min and the activation was reversed within 15 min upon its removal (57). Furthermore, this rapid activation was independent of de novo synthesis of the Na^+-pump and thus could be mediated by rapid insertion of the Na^+-pump to the basolateral membrane recruited from its intracellular pool (56, 57).

In the proximal renal tubules, the number of the Na^+-pump in the basolateral membrane is known to be modulated. Activation of PKA and phosphorylation of Ser-943 of the α-catalytic subunit and/or dephosphorylation of unknown amino acid residue by the Ca^{2+}-calmodulin dependent protein phosphatase 2B (calcineurin) was shown to increase the number of the Na^+-pump by exocytic insertion of the pump (58, 59). Conversely, an activation of PKC via dopamine was found to phosphorylate Ser-11 and Ser-18 of the α-catalytic subunit and led to inhibit Na^+-pump activity within 1-2 min by triggering clathrine-dependent endocytosis (60, 61). The endocytosis of the Na^+-pump was dependent upon actin microfilaments and microtubules (60). Thus, current evidence points to the importance of the regulation of Na^+-pump activity by reversible exocytic insertion into and endocytic retrieval from the basolateral membrane.

PERSPECTIVES

This review highlighted the vesicular transport as an important and perhaps generalized mechanism for the short term regulation of the transepithelial water and electrolyte transport in the renal epithelium. The underlying molecular mechanisms for exocytic insertion into and endocytic retrieval of transporter-carrying vesicles remain largely unknown and under intensive investigation. Among known characteristics common to the vesicular transport is a close association with a change in the cell volume. Hypotonic cell swelling was associated with or required for an increase in the number of transporters in the plasma membrane, including H^+-ATPase (62), NHE-3 (63), Npt2 (64), ENaC (65) and Na^+-pump (66). Changes in cell volume per se have been considered as cellular messages mediating cellular response to stimuli (67). Most, if not all, exocytic and endocytic vesicles have an acidic intravesicular pH by an electrogenic vacuolar H^+-ATPase in the vesicle membrane (32, 68). Active vacuolar H^+-ATPase is required for both endocytic and exocytic processes (33, 69, 70). It is specially worthy to note that an inhibition of vacuolar H^+-ATPase with bafilomycin A1 has most recently shown to inhibit exocytic insertion of AQP2 in response to vasopressin (71). Furthermore, cell swelling was found to lead to cytosolic acidification concomitantly with vacuolar alkalinization (67). The vacuolar H^+-ATPase, being electrogenic, is likely to be activated. In addition, the intracellular vesicle membrane is known to have water channels (35) and Cl^- channels (72-74). These findings, taken together, point to the possibility that cell swelling might activate the vacuolar H^+-ATPase in the transporter-carrying vesicle membrane through cytosolic acidification, i.e., providing H^+ to the pump. The activated H^+-ATPase would induce Cl^- influx into the vesicle driven by a positive electrical potential, resulting in chemiosmotic swelling of the vesicle as a result of H_2O influx obligated with intravesicular HCl accumulation.

By inference from author's chemiosmotic hypothesis for the control of exocytic release of renin (33, 34), chemiosmotic swelling of renin secretory granules is postulated to induce fusion of renin secretory granule membrane with the plasma membrane. In a similar manner, chemiosmotic swelling of transporter-carrying vesicles may lead to exocytic insertion of transporter into the plasma membrane, thereby increasing the abundance of transporter and stimulating transepithelial water and electrolyte transport.

In this review, the possibility that an involvement of vesicular transport in the short-term regulation of only five representative transporters was discussed. But many other transporters such as cystic fibrosis transmembrane conductance regulator (CFTR) Cl^--channel, Na^+, K^+, $2Cl^-$ cotransporter and vasopressin-sensitive urea transporter are also likely to be regulated by vesicular trans-

port. The pathophysiology of various renal diseases including Liddle's syndrome, Dent's disease, nephrogenic diabetes insipidus and polycystic kidney disease can be understood in terms of derangement of vesicular transport. Thus, this field of research would be the subject of intensive investigation in future.

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