A possible catalytic role for NH₄⁺ in Na⁺ reabsorption across the thick ascending limb

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Active reabsorption of Na⁺ across the thick ascending limb of Henle’s loop (TALH) plays an essential role in the urine concentrating mechanism, as it generates and maintains the axial osmolality gradient in the outer medulla. Na⁺ enters the TALH cells mostly through apical Na⁺-K⁺-2Cl⁻ (NKCC2) cotransporters, to a lesser extent through Na⁺/H⁺ (NHE3) exchangers, and it is extruded at the basolateral membrane via Na⁺-K⁺-ATPase pumps. The recycling of K⁺ at the luminal membrane, via ROMK channels, is necessary to prevent luminal K⁺ depletion; luminal K⁺ is therefore said to be catalytic for Na⁺ reabsorption. Cl⁻ exits the cell via basolateral K⁺-Cl⁻ (KCC) cotransporters and Cl⁻ channels. The fact that the predominant TALH transporters involved in Na⁺ transport (viz. NKCC2, KCC, Na⁺-K⁺-ATPase) can carry NH₄⁺ in lieu of K⁺ suggests that Na⁺ reabsorption may be linked to acid-base metabolism, but this coupling is seldom acknowledged in standard descriptions of Na⁺ transport across the TALH.

In a current series of reports in *The American Journal of Physiology-Renal Physiology* (15–17), Weinstein presents a sophisticated mathematical model of ionic transport across the TALH. A novel and important finding of the new model is that luminal NH₄⁺ may play a catalytic role, quantitatively comparable to that of K⁺, for Na⁺ reabsorption. In the first of three companion studies (15), Weinstein developed kinetic models for two key TALH transporters, NKCC2 and KCC4. Model parameters were fitted to flux measurements reported in the literature, and the experimental data for NKCC2 were adequately reproduced when similar binding affinities for K⁺ and NH₄⁺, but lower translocation rates for NH₄⁺-loaded carrier, were assumed. The NKCC2 and KCC4 representations were subsequently incorporated into a model of the TALH epithelial cell (17) and of the entire tubule (16) so as to simulate TALH function in vitro and in vivo. The only prior TALH model, developed by Fernandes and Ferreira (2), applied to one cell and did not incorporate acid-base metabolism.

What is the theoretical basis for the catalytic role of NH₄⁺? Measured NH₄⁺ and K⁺ concentrations in the TALH lumen are similar (8), whereas the cytosolic concentration of NH₄⁺ is likely to be lower than that of K⁺. Apical transmembrane ionic gradients are therefore more favorable to Na⁺-NH₄⁺-2Cl⁻ entry via NKCC2 than to Na⁺-K⁺-2Cl⁻ entry. Thus, assuming comparable K⁺ and NH₄⁺ affinities for the transporter, the TALH model of Weinstein predicts substantial NH₄⁺ entry through NKCC2. The calculated rates of luminal NH₄⁺ uptake are compatible with experimental measurements of intracellular pH variations upon addition of external NH₄Cl (6). The calculated rates can also be reconciled with the experimental finding of low TALH ammonia reabsorption (5), provided that there is significant recycling of NH₄⁺ across the luminal membrane. The mechanisms underlying luminal NH₄⁺ backflux nevertheless remain unclear. Since neither ROMK nor NHE3 appears to transport large amounts of NH₄⁺, the model posits significant diffusive NH₄⁺ exit in parallel with H⁺ secretion via NHE3. However, as acknowledged by the investigator, predicted proton secretion rates are difficult to reconcile with the low rates of HCO₃⁻ absorption in rat TALH perfused in vitro (4, 5). Whether luminal NHE3 activity is sufficient to sustain these proton fluxes also remains uncertain.

The current reports by Weinstein (15–17) appear to be the first studies to suggest a catalytic role for NH₄⁺ in TALH Na⁺ reabsorption, and carefully designed experimental studies are required to confirm this theoretical finding. In particular, measurements are needed to determine the relative fluxes of NH₄⁺ and K⁺ through NKCC2 under physiological conditions, and whether the activity of NHE3 in TALH is large enough to allow for significant luminal NH₄⁺ recycling. The prediction that both K⁺ and NH₄⁺ are catalytic for TALH Na⁺ reabsorption also raises questions as to why these two overlapping mechanisms should coexist. HCO₃⁻ reabsorption by the TALH appears to be regulated so as to support parallel increases in NH₄⁺ reabsorption (10, 12). It is possible that among the factors that stimulate HCO₃⁻ transport (3, 14), some, such as hypokalemia (1), also modulate the activity of NKCC2 by altering the balance between K⁺-dependent and NH₄⁺-dependent Na⁺ reabsorption.

The TALH modeling studies are also important in the context of the urine concentrating mechanism. The tubular model (16) focuses on the modulation of Na⁺ transport by K⁺, and by transporter defects akin to those found in Bartter syndrome. It validates the hypothesis, first proposed by Stokes (13), that accumulation of K⁺ in the outer medullary interstitium, by reducing NaCl absorption across the TALH, increases Na⁺ and water delivery to the distal nephron, and thereby amplifies cortical K⁺ secretion. The TALH model suggests that peritubular K⁺ concentration can modulate KCC4 activity and subsequently Na⁺ entry via NKCC2, with cytosolic Cl⁻ acting as a mediator in the peritubular-to-luminal membrane cross talk. The model also shows that increases in peritubular K⁺ alkalize the TALH cell, thereby affecting luminal NHE3 activity. Thus peritubular K⁺ is predicted to modulate both luminal pathways for Na⁺ entry. Models of the urine concentrating mechanisms generally represent only the transport of water, Na⁺, and urea, and assume fixed transepithelial permeabilities for a given tubular segment (11, 18). The TALH model results suggest that they fail to capture coupling mechanisms which may be significant in the formation of concentrated urine.
Although not a present focus of the study, the TALH model could also help to resolve long-standing questions related to the importance of paracellular Na\(^+\) transport across the TALH in vivo and medullary oxygen requirements. Typical representations of NaCl transport across the TAL posit that for each Na\(^+\) ion actively transported through the cell, one Na\(^+\) is transported through the paracellular pathway without additional energy expenditure (7). This scheme has been challenged by Kiil and Sejersted (9), who noted that under in vitro conditions, peritubular and luminal Na\(^+\) concentrations are typically equal, whereas in vivo, the interstitial-to-lumen Na\(^+\) concentration ratio is generally much higher than 1. Thus the transepithelial concentration gradient and the lumen-positive electrical potential difference exert counterbalancing effects on the paracellular transport of Na\(^+\). Measurements of energy expenditure, i.e., the ratio of NaCl reabsorbed to oxygen consumption, suggest that in vivo all Na\(^+\) transport along TAHL is active (9). The tubular study of Weinstein (16) does not explicitly display predictions of paracellular vs. transcellular Na\(^+\) fluxes in vivo, but the in vitro results suggest that the net Na\(^+\) flux is approximately all transcellular. This new TALH model raises intriguing questions that must be investigated both experimentally and theoretically.

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DISCLOSURES

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REFERENCES