The Physiology of Urinary Concentration: An Update

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Summary: The renal medulla produces concentrated urine through the generation of an osmotic gradient extending from the cortico-medullary boundary to the inner medullary tip. This gradient is generated in the outer medulla by the countercurrent multiplication of a comparatively small transepithelial difference in osmotic pressure. This small difference, called a single effect, arises from active NaCl reabsorption from thick ascending limbs, which dilutes ascending limb flow relative to flow in vessels and other tubules. In the inner medulla, the gradient may also be generated by the countercurrent multiplication of a single effect, but the single effect has not been definitively identified. There have been important recent advances in our understanding of key components of the urine concentrating mechanism. In particular, the identification and localization of key transport proteins for water, urea, and sodium, the elucidation of the role and regulation of osmoprotective osmolytes, better resolution of the anatomical relationships in the medulla, and improvements in mathematic modeling of the urine concentrating mechanism. Continued experimental investigation of transepithelial transport and its regulation, both in normal animals and in knock-out mice, and incorporation of the resulting information into mathematic simulations, may help to more fully elucidate the inner medullary urine concentrating mechanism.
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The mammalian kidney maintains nearly constant blood plasma osmolality and nearly constant blood plasma sodium concentration by means of mechanisms that independently regulate water and sodium excretion. Because many mammals do not have continuous access to water, the ability to vary water excretion can be essential for survival. Because sodium and its anions are the principal osmotic constituents of blood plasma, and stable electrolyte concentrations are also essential, water excretion must be regulated by mechanisms that decouple it from sodium excretion.

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The urine concentrating mechanism plays a fundamental role in regulating water and sodium excretion. When water intake is large enough to dilute blood plasma, a urine more dilute than blood plasma is produced; when water intake is so small that blood plasma is concentrated, a urine more concentrated than blood plasma is produced. In both cases, the total urinary solute excretion rate and the urinary sodium excretion rate are small and normally vary within narrow bounds.

In contrast to solute excretion, urine osmolality varies widely in response to changes in water intake. After several hours without water intake, such as occurs overnight during sleep, human urine osmolality may increase to approximately 1,200 mOsm/kg H_2O, about 4 times plasma osmolality (~290 mOsm/kg H_2O). Conversely, urine osmolality may decrease rapidly after the ingestion of large quantities of water, such as commonly occurs at breakfast, at which point human urine osmolality (and that of other mam-

mals) may decrease to approximately 50 mOsm/kg H_2O. Most physiologic studies relevant to the urine concentrating mechanism have been conducted in species that can achieve higher maximum urine osmolalities than human beings. For example, rabbits can concentrate to approximately 1,400 mOsm/kg H_2O, rats to approximately 3,000 mOsm/kg H_2O, mice and hamsters to approximately 4,000 mOsm/kg H_2O, and chinchillas to approximately 7,600 mOsm/kg H_2O (reviewed by us previously[1]).

All mammalian kidneys maintain an osmotic gradient that increases from the corticomedullary boundary to the tip of the medulla (papillary tip). This osmotic gradient is sustained even in diuresis, although its magnitude is diminished relative to antidiuresis.[2,3] NaCl is the major constituent of the osmotic gradient in the outer medulla, whereas NaCl and urea are the major constituents in the inner medulla.[2,3] The cortex is nearly isotonic to plasma, whereas the inner medullary (papillary) tip is hypertonic to plasma, and has osmolality similar to urine during antidiuresis.[4] Sodium and potassium, accompanied by univalent anions, and urea are the major urinary solutes; urea is normally the predominant urinary solute during a strong antidiuresis.[2,3]

The mechanisms for the independent control of water and sodium excretion are contained mostly within the renal medulla. The medullary nephron segments and vasa recta are arranged in complex but specific anatomic relationships, both in terms of 3-dimensional configuration and in terms of which segments connect to which segments. The production of concentrated urine involves complex interactions among the medullary nephron segments[5,6] and vasculature. In outer medulla, the thick ascending limbs of the loops of Henle actively reabsorb NaCl. This serves 2 vital functions: it dilutes the luminal fluid, and it provides NaCl to increase the osmolality of the medullary interstitium, pars recta, descending limbs, vasculature, and collecting ducts. Both the nephron segments and vessels are arranged in a countercurrent configuration, thereby facilitating the generation of a medullary osmolality gradient along the corticomedullary axis. In inner medulla, osmolality continues to increase, although the source of the concentrating effect remains controversial. The most widely accepted mechanism remains the passive reabsorption of NaCl, in excess of solute secretion, from the thin ascending limbs of the loops of Henle.[7,8]

Perfused tubule studies provided the basis for many of the theories of how concentrated urine is produced (reviewed by us previously[1]). The cloning of many of the proteins that mediate urea, sodium, and water transport in nephron segments that are important for urinary concentration and dilution have provided additional insights into the urine concentrating mechanism (Fig. 1). In general, the urea, sodium, and water transport proteins are highly specific and appear to eliminate a molecular basis for solvent drag; this specifically suggests that the reflection coefficients should be 1.[1] For a detailed review of these transport properties, the reader is referred to our previous report.[1]

GENERAL FEATURES OF THE CONCENTRATING MECHANISM

Countercurrent Multiplication

Countercurrent multiplication refers to the process by which a small osmolality difference, at each level of the outer medulla, between fluid flows in ascending and descending limbs of the loops of Henle, is multiplied by the countercurrent flow configuration to establish a large axial osmolality difference. This axial difference frequently is referred to as the *corticomedullary osmolality gradient* because it is distributed along the corticomedullary axis. Figure 2 illustrates the principle of countercurrent multiplication. Figure 2 shows a schematic of a short loop of Henle: the left channel represents the descending limb and the right channel represents the thick ascending limb. A water-impermeable barrier separates the 2 channels. Vertical arrows indicate flow down the left channel and up the right channel. Horizontal arrows (left-directed) indicate active transport of solute from the right channel to the left channel. Local fluid osmolality is indicated by the numbers within the channels. Successive panels represent the time course of the multiplication process.

Figure 1. Molecular identities and locations of the sodium, urea, and water transport proteins involved in the passive mechanism hypothesis for urine concentration in the inner medulla.[7,8] The major kidney regions are indicated on the left. NaCl is actively reabsorbed across the thick ascending limb by the apical plasma membrane Na-K-2Cl cotransporter (NKCC2/BSC1), and the basolateral membrane Na/K–adenosine triphosphatase (not shown). Potassium is recycled through an apical plasma membrane channel, ROMK. Water is reabsorbed across the descending limb segments by AQP1 water channels in both apical and basolateral plasma membranes. Water is reabsorbed across the apical plasma membrane of the collecting duct by AQP2 water channels in the presence of vasopressin. Water is reabsorbed across the basolateral plasma membrane by AQP3 water channels in the cortical and outer medullary collecting ducts and by both AQP3 and AQP4 water channels in the IMCD. Urea is concentrated within the collecting duct lumen (by water reabsorption) until it reaches the terminal IMCD where it is reabsorbed by the urea transporters UT-A1 and UT-A3. According to the passive mechanism hypothesis (see text), the fluid that enters the thin ascending limb from the contiguous thin descending limb has a higher NaCl and a lower urea concentration than the inner medullary interstitium, resulting in passive NaCl reabsorption and dilution of the fluid within the thin ascending limb. UT, urea transporter; AQP, aquaporin.

The schematic loop starts with isosmolar fluid throughout (Fig. 2A). In Figure 2B, enough solute has been pumped by an active transport mechanism to establish a 20-mOsm/kg H_2O osmolality difference between the ascending and descending flows at each level. This small osmolality difference, transverse to the flow, is called the *single effect*. Osmolality values after the fluid has convected the solute halfway down the left channel and halfway up the right channel are illustrated in Figure 2C. In Figure 2D, a 20-mOsm/kg H_2O osmolality difference has been re-established by the active transport mechanism, and the luminal fluid near the bend of the loop has attained a higher osmolality than in Figure 2A. A progressively higher osmolality is attained at the loop bend by successive iterations of this process. A large osmolality difference is generated along the flow direction, as illustrated in Figure 2E, where the osmolality at the loop bend is nearly 300 mOsm/kg H_2O above the osmolality of the fluid entering the loop. Thus, a 20-mOsm/kg H_2O difference, the single effect, has been multiplied axially down

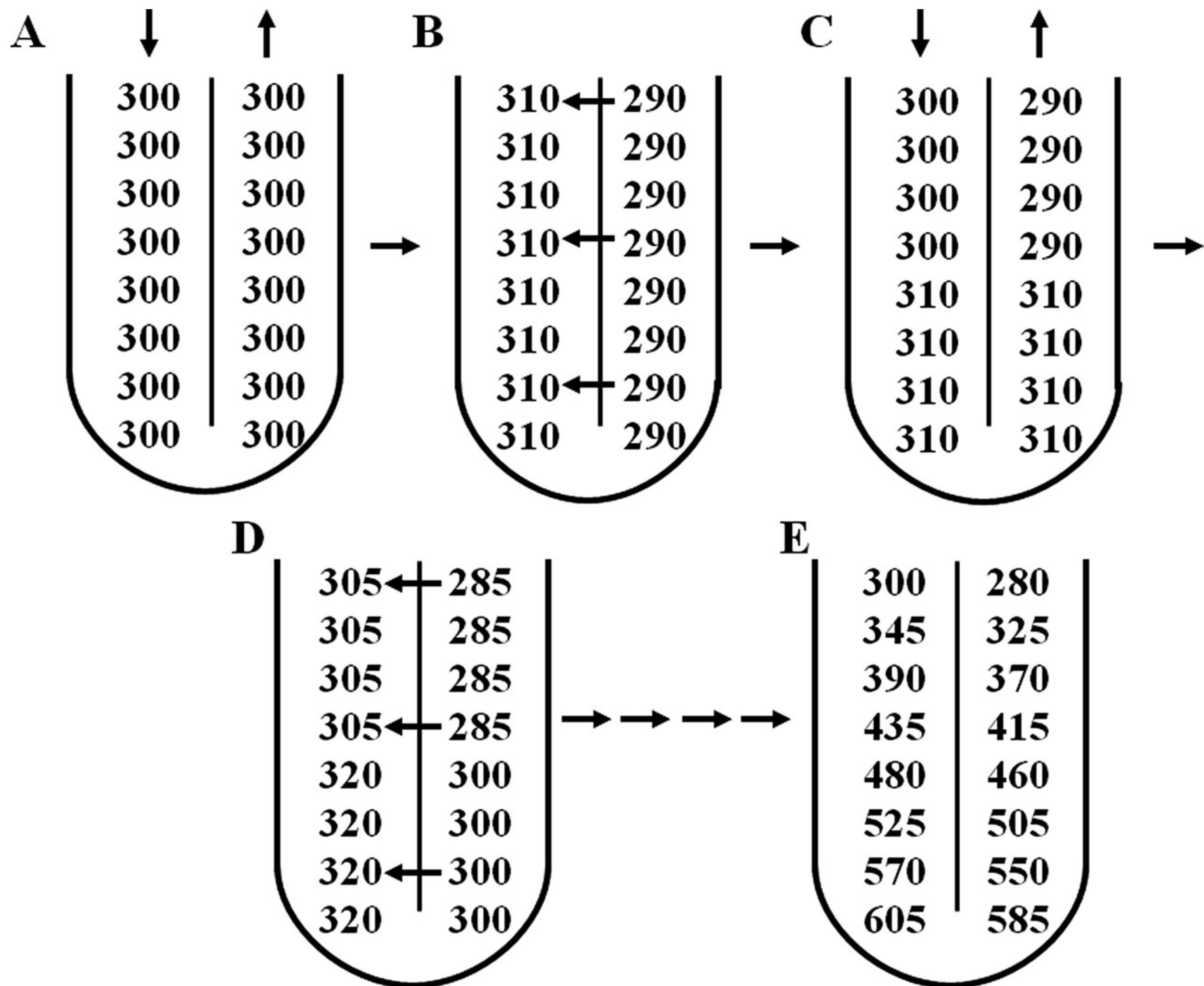

Figure 2. Countercurrent multiplication of a single effect in a diagram of the loop of Henle in the outer medulla. (A) Process begins with isosmolar fluid throughout both limbs. (B) Active solute transport establishes a 20-mOsm/kg H_2O transverse gradient (single effect) across the boundary separating the limbs. (C) Fluid flows halfway down the descending limb and up the ascending limb. (D) Active transport reestablishes a 20-mOsm/kg H_2O transverse gradient. Note that the luminal fluid near the bend of the loop achieves a higher osmolality than loop-bend fluid in panel B. (E) As the processes in C and D are repeated, the bend of the loop achieves a progressively higher osmolality so that the final axial osmotic gradient far exceeds the transverse 20-mOsm/kg H_2O gradient generated at any level.

the length of the loop by the process of countercurrent multiplication.

In short loops of Henle, the process of countercurrent multiplication is similar to the process shown in Figure 2. The tubular fluid emerging from the end of the proximal tubule and entering the outer medulla is isotonic to plasma (~290 mOsm/kg H_2O). That tubular fluid is concentrated as it passes through the proximal straight tubule (pars recta) and on into the thin descending limb of the loop of Henle. The tubular fluid osmolality attains an osmolality about twice that of blood plasma at the bend of the loop of Henle. The fluid then is diluted as it flows up the medullary thick ascending limb of the loop of Henle, so that the tubular fluid emerging from this nephron segment is hypo-osmotic to plasma. The thick ascending limb is nearly impermeable to water and no aquaporin proteins have been detected in this nephron segment (reviewed by us previously[1]). The thick ascending limb has a low NaCl permeability, but it vigorously transports NaCl from the tubular lumen to the medullary interstitium by an active transport mechanism.

Countercurrent Exchange

The blood circulation to the medulla, which is supplied by the descending and ascending vasa recta, is arranged in a counterflow configuration connected by a capillary plexus. Vasa recta achieve osmotic equilibration through a combination of water and solute transport because they are freely permeable to water, urea, and sodium.[9] Descending vasa recta lose water and gain solute, whereas ascending vasa recta gain water and lose solute. The exchange of water and solute between the descending and ascending vasa recta and the surrounding interstitium is called *countercurrent exchange*.

Countercurrent exchange must be highly efficient to produce a concentrated urine because hypotonic fluid carried into the medulla and hypertonic fluid carried away from the medulla will each tend to dissipate the work of countercurrent multiplication. Thus, fluid flowing through the vasa recta must achieve near-osmotic equilibrium with the surrounding interstitium at each medullary level, and fluid entering the cortex from the ascending vasa recta must have an osmolality close to that of blood plasma, to minimize wasted work. Conditions that decrease medullary blood flow, such as volume depletion, improve urine concentrating ability and the efficiency of countercurrent exchange by allowing more time for blood in the ascending vasa recta to lose solute and achieve osmotic equilibration.[9] Conversely, conditions that increase medullary blood flow, such as osmotic diuresis, decrease urine concentrating ability and impair the efficiency of countercurrent exchange.[9] For a more detailed treatment of countercurrent exchange, the reader is referred to the article by Pallone et al.[10]

URINE CONCENTRATING MECHANISM: HISTORY AND THEORY

Overview

One may divide the conceptual history of the concentrating mechanism into 3 periods. The first period (1942-1971) was inaugurated by a study by Kuhn and Ryffel[11] that proposed that the production of a concentrated urine results from the countercurrent multiplication of a "single effect." Kuhn and Ryffel[11] constructed a working apparatus that exemplified the principles of countercurrent multiplication. This first period saw the further development of the theory of the countercurrent multiplication hypothesis and the generation of experimental evidence that supported the hypothesis as the explanation for the urine concentrating mechanism of the outer medulla.[12] In particular, active transport of NaCl from thick ascending limbs of the loops of Henle was identified as the source of the outer medullary single effect.[13,14]

The second period (1972-1992) was inaugurated by the simultaneous publication of 2 seminal reports, one by Kokko and Rector[7] and one by Stephenson,[8] proposing that a "passive" mechanism provides the single effect for countercurrent multiplication in the inner medulla. According to the passive mechanism hypothesis, a net solute efflux from thin ascending limbs of the loops of Henle results from favorable transepithelial urea and NaCl gradients; these gradients arise from the separation of urea and NaCl, which is driven by the outer medullary concentrating mechanism.

Although a large body of experimental evidence initially appeared to support the passive mechanism, findings from several subsequent studies are difficult to reconcile with this hypothesis.[15-17] Moreover, when the measured transepithelial permeabilities were incorporated into mathematic models, the models failed to predict a significant inner medullary concentrating effect.[18-20] The discrepancy between the very effective inner medullary concentrating effect and the consistently negative results from mathematic modeling studies has persisted through more than 3 decades. The discrepancy has helped to stimulate the formulation of several highly sophisticated mathematic models (notably the model by Wexler et al[21]) and research on the transport properties of the renal tubules of the inner medulla, but no model study has resolved the discrepancy to the general satisfaction of modelers and experimentalists.

A third period of conceptual thought may be considered to have begun in 1993 as new hypotheses for the inner medullary concentrating mechanism began to receive serious consideration. In 1993, a key role for the peristalsis of

the papilla was proposed by Chou et al[16] and Knepper et al.[22] In 1994, the principle of "externally driven countercurrent multiplication,"[23] arising, for example, by the net production of osmotically active particles in the interstitium, was considered by Jen and Stephenson. At about the same time, experimental measurements in perfused tubules from chinchillas, which can produce very highly concentrated urine, provided evidence that the passive mechanism, as originally proposed, cannot explain the inner medullary urine concentrating mechanism.[24] Recent studies have sought to further develop hypotheses involving the potential generation of osmotically active particles, especially lactate,[25,26] and peristalsis of the papilla.[27] In 2004, hypotheses related to the passive mechanism were reconsidered because of experimental evidence suggesting an absence of significant urea transport proteins in loops of Henle reaching deep into the inner medulla.[28] Recently, Pannabecker et al[5] proposed that the spatial arrangements of loop of Henle subsegments and the identification of multiple countercurrent systems in the inner medulla, along with their initial mathematic model, are most consistent with a solute-separation, solute-mixing mechanism for the inner medullary urine concentrating mechanism.

Urine Concentrating Mechanism in the Outer Medulla

The urine concentrating mechanism is believed to operate as follows in the outer medulla. NaCl actively is transported from the tubular fluid of thick ascending limbs of the loops of Henle into the surrounding interstitium, mediated by the Na-K-2Cl cotransporter NKCC2/BSC1 in the apical plasma membrane and Na-K-adenosine triphosphatase in the basolateral plasma membrane. This active NaCl reabsorption increases the osmolality of interstitial fluid and promotes the osmotic reabsorption of water from the tubular fluid of descending limbs and collecting ducts. Because of the reabsorption of fluid from descending limbs of the loops of Henle, the fluid delivered to the ascending limbs has a high NaCl concentration that favors transepithelial NaCl transport from ascending limb fluid. (There also may be some NaCl diffusion into descending limb fluid.) NaCl reabsorption dilutes the thick ascending limb tubular fluid, so that at each medullary level the fluid osmolality is less than that in the other tubules and vessels, and so that the fluid delivered to the cortex is dilute relative to blood plasma. The ascending limb fluid that enters the cortex is diluted further by active NaCl reabsorption from cortical thick ascending limbs, so that its osmolality is less than the osmolality of blood plasma. In the presence of vasopressin (antidiuretic hormone), cortical collecting ducts are highly water-permeable, and sufficient water is reabsorbed to return the fluid to isotonicity with blood plasma. This cortical water reabsorption greatly reduces the load that is placed on the urine concentrating mechanism by the fluid that re-enters the medulla via the collecting ducts. In the absence of vasopressin, the entire collecting duct system has limited water permeability, and even though some water is reabsorbed because of the very large osmotic pressure gradient, fluid that is dilute relative to plasma is delivered by the collecting ducts to the border of the outer and inner medulla.

This modern conceptual formulation of the outer medullary urine concentrating mechanism (which is very similar to the proposal of Hargitay and Kuhn[29] as modified by Kuhn and Ramel[30]) is supported by recent mathematic modeling studies using parameters compatible with perfused tubule and micropuncture experiments (reviewed by us previously[1]). In particular, the outer medullary osmotic gradients predicted by mathematic simulations[31,32] are consistent with the gradients reported in tissue slice experiments, in which osmolality is increased by a factor of 2 to 3.[31-34]

The Passive Mechanism Hypothesis for the Inner Medulla

In contrast to the outer medulla, with active NaCl transport from thick ascending limbs generating the single effect, isolated perfused tubule experiments in rabbit thin ascending limbs showed no significant active NaCl transport.[13,35] Instead, the thin ascending limb had relatively high permeabilities to sodium and urea while being impermeable to water.[36] In contrast, the inner medullary thin descending limb is highly

water-permeable but has low urea and sodium permeabilities.[37,38] Moreover, it had long been known that urea administration enhances maximum urine concentration in protein-deprived rats and human beings,[39] and evidence from some species showed that urea tended to accumulate in the inner medulla, with concentrations similar to those of NaCl.[3] Several inner medullary concentrating mechanism models were published that failed to gain general acceptance (reviewed by us previously[1]).

In 1972, there were 2 independent reports, one by Kokko and Rector[7] and one by Stephenson[8] (appearing in the same issue of *Kidney International*), that proposed that the single effect in the inner medulla arises from a "passive mechanism." The urea concentration of collecting duct fluid is increased by active absorption of NaCl from the thick ascending limb and the subsequent absorption of water from the cortical and outer medullary collecting ducts. In the highly urea permeable terminal inner medullary collecting duct (IMCD), urea diffuses down its concentration gradient into the inner medullary interstitium; urea is trapped in the inner medulla by countercurrent exchange in the vasa recta. Fluid entering thin ascending limbs has a high NaCl concentration relative to urea, and the thin ascending limb is hypothesized to have a high NaCl permeability relative to urea. In addition, because of inner medullary interstitial accumulation of urea, the NaCl concentration in the thin ascending limb exceeds the NaCl concentration in the interstitium, and consequently NaCl diffuses down its concentration gradient into the interstitium. If the urea permeability of the thin ascending limb is sufficiently low, the rate of NaCl efflux from the thin ascending limb will exceed the rate of urea influx, resulting in dilution of thin ascending limb fluid and the flow of relatively dilute fluid up the thin ascending limb at each level and into the thick ascending limb. Thus, dilute fluid is removed from the inner medulla, as required by mass balance, and the interstitial osmolality is increased progressively along the tubules of the inner medulla. Water is drawn from the thin descending limbs by the increased osmolality, thus increasing the NaCl concentration of the descending limb flow that enters thin ascending limbs. In addition, the increased osmolality of the inner medullary interstitium draws water from the water-permeable IMCD, increasing the concentration of urea in collecting duct fluid; accumulation of NaCl in the interstitium tends to sustain a transepithelial urea concentration gradient favorable to urea reabsorption from the terminal IMCD.

Several matters regarding the passive mechanism merit discussion. First, this process should be thought of as a continuous, steady-state process, even though it has been described in a step-wise fashion. Second, even though the mechanism is characterized as passive, it depends on the separation of urea and NaCl that is sustained by active NaCl reabsorption by thick ascending limbs. The separated high-concentration flows of NaCl (in the loops of Henle) and of urea (in the collecting ducts) constitute a source of potential energy that is used to effect a net transport of solute from the thin ascending limbs. Thus, there is no violation of the laws of thermodynamics. Third, the earlier description speaks rather loosely of NaCl and urea as solutes having equal standing, but NaCl nearly completely is dissociated into Na and Cl ions, so that each NaCl molecule has nearly twice the osmotic effect of each urea molecule. Formal mathematic descriptions must represent this distinction. Fourth, the passive mechanism hypothesis is very similar to the outer medullary urine concentrating mechanism inasmuch as it depends on net solute absorption from the thin ascending limb to dilute thin ascending limb fluid and increase the osmolality in vasa recta and collecting ducts. Thus, the production of a small amount of highly concentrated urine is balanced by a larger amount of slightly dilute flow in the thin ascending limbs. Although the osmolality gradient along the inner medulla depends on countercurrent exchange, especially exchange between descending and ascending vasa recta, equilibration in countercurrent flows is incomplete. Hence the achievable urine osmolality is limited by the dissipative effect of ascending flows that are slightly concentrated relative to descending flows.

Critique and Re-emergence of the Passive Mechanism Hypothesis

The passive mechanism hypothesis, as described earlier, closely follows the Kokko and Rector[7] formulation, which made use of key ideas in a largely experimental study by Kokko.[38] Kokko and Rector[7] acknowledged Niesel and Röskenbleck[40] for the idea that IMCD urea reabsorption contributes to the inner medullary osmolality gradient. Kokko and Rector[7] presented a conceptual model of the passive mechanism hypothesis and, although it was accompanied by a plausible set of solute fluxes, concentrations, and fluid flow rates that are consistent with the requirements of mass balance, it did not show that measured loop of Henle permeabilities were consistent with the hypothesis, and their presentation did not include a mathematic treatment. Stephenson's[8] formulation of the passive mechanism hypothesis introduced the highly influential central core assumption and included a more mathematic treatment, but it also did not contain a mathematic reconciliation of tubular transport properties with the hypothesis.

In recent years, mathematic simulations of the urine concentrating mechanism have become increasingly comprehensive and sophisticated in the representation of medullary architecture[18,21,41-43] and tubular transport.[44-46] This evolution is a consequence of faster computers with increased computational capacity, the increasing body of experimental knowledge, and the sustained failure of simulations to show a significant inner medullary concentration gradient.

Studies by Pannabecker et al,[5] conducted by means of immunohistochemical labeling and computer-assisted reconstruction, have revealed much new detail about the functional architecture of the rat inner medulla. In particular, their findings indicated that descending thin limbs (DTLs) of loops of Henle turning within the upper first millimeter of the inner medulla do not have significant aquaporin-1 (AQP1), whereas DTLs of loops turning below the first millimeter have 3 discernible functional subsegments: the upper 40% of these DTLs express AQP1, whereas the lower 60% do not; moreover, the final approximately 165 μm expresses ClC-K1, as does the contiguous thin ascending limb (Fig. 3).

Layton et al[28] recently proposed 2 hypotheses closely related to the passive mechanism; these hypotheses were motivated by implications of recent studies in rats by Pannabecker et al.[47,48] One hypothesis is based directly on principles of the passive mechanism: thin limbs of loops of Henle were assumed to have low urea permeabilities because no significant labeling for urea transport proteins was found in loops reaching deep into the inner medulla.[28] A second, more innovative hypothesis assumed very high loop of Henle urea permeabilities, but limited NaCl permeability and zero water permeability in thin descending limbs reaching deep into the inner medulla. Thus, in the deepest portion of the inner medulla, tubular fluid urea concentration in loops of Henle would nearly equilibrate with the local interstitial urea concentration; thin descending limb fluid osmolality would be increased by urea secretion; and substantial NaCl reabsorption would occur in the prebend segment and early thin ascending limb. The role of the decreasing loop of Henle population is emphasized in both hypotheses, which facilitates a spatially distributed NaCl reabsorption along the inner medulla, from prebend segments and early thin ascending limbs. A distinctive aspect of both hypotheses is an emphasis on NaCl reabsorption from the IMCDs as an important active transport process that separates NaCl from tubular fluid urea and that indirectly drives water and urea reabsorption from the collecting ducts. Computer simulations for both hypotheses predicted urine flow, concentrations, and osmolalities consistent with urine from moderately antidiuretic rats. The first hypothesis has a critical dependence on low loop of Henle urea permeabilities and is subject to the criticism that urea transport may be paracellular rather than transepithelial: that hypothesis depends on more conclusive experiments to determine urea transport properties in the rat. The second hypothesis may contribute to understanding the chinchilla urine concentrating mechanism, in which high loop urea permeabilities have been measured.[24]

Alternatives to the Passive Mechanism

Alternatives to the original passive mechanism hypothesis fall into 3 categories. First, many

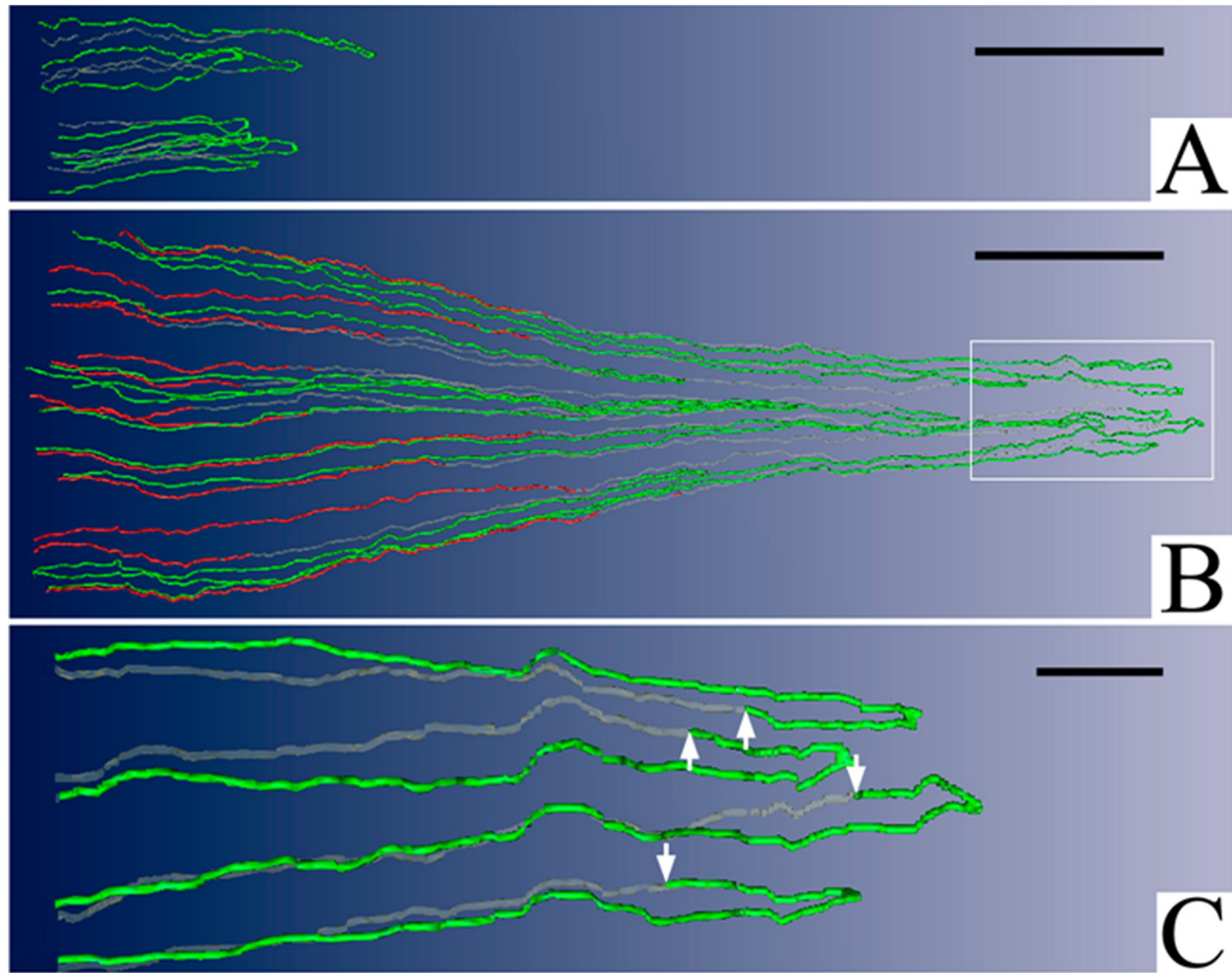

Figure 3. Reconstruction of loops of Henle from rat inner medulla. Red indicates expression of AQP1; green indicates ClC-K1; and gray indicates both AQP1 and ClC-K1 are undetectable. (A) Loops that turn within the first millimeter beyond the outer medulla. DTLs lack detectable AQP1; ClC-K1 is expressed along prebend segments and ascending thin limbs. (B) Loops that turn beyond the first millimeter of the inner medulla. DTLs express AQP1 along the initial approximately 40%; ClC-K1 is expressed along the prebend segments and ATLs. (C) Enlargement of near-bend regions from boxed area in panel B. Prebend ClC-K1 expression, on average, begins approximately 165 μm before the loop bend (arrows). Scale bars: (A and B) 500 μm; (C) 100 μm. ATL, ascending thin limb; DTL, descending thin limb; AQP1, aquaporin-1; ClC-K1, chloride channel K1. Reprinted with permission from Pannabecker et al[5] and the American Physiological Society.

simulation studies have attempted to show that a better representation of medullary anatomy or transepithelial transport is required for the effective operation of the passive mechanism. Second, a number of steady-state mechanisms involving a single effect generated in either collecting ducts or thin descending limbs have been proposed. Third, several hypotheses have been proposed that depend on the peristaltic contractions of the pelvic wall, and their impact on the papilla. A detailed discussion of the steady-state alternatives involving collecting ducts or thin descending limbs can be found in our previous article.[1]

Schmidt-Nielsen[49] proposed a hypothesis that depends on the peristaltic contractions of the pelvic wall: the contraction-relaxation cycle creates negative pressures in the interstitium that act to transport water, in excess of solute, from the collecting duct system. According to this hypothesis, the compression wave would increase hydrostatic pressure in the collecting duct lumen, promoting a water flux into collecting duct cells. Water flow through aqua-

porin water channels would be induced by the pressure without a commensurate solute flux. Thus, the remaining luminal fluid would be concentrated, relative to the contents of collecting duct cells and the surrounding interstitium. After passage of the peristaltic wave, the collecting ducts would be collapsed. The papilla, transiently narrowed and lengthened by the wave, would rebound and a negative hydrostatic pressure would develop in the elastic interstitium, which is rich in glycosamine glycans and hyaluronic acid. Water would be withdrawn from the collecting duct cells (through aquaporins) by the negative pressure and enter into the vasa recta, which re-open during the relaxation phase of the contraction and carry reabsorbate toward the cortex. This hypothesis appears to provide no role for long loops of Henle or the special role of urea in producing concentrated urine,[39] and it does not explain the large NaCl gradient generated in the papilla.[3,50]

Knepper et al[27] recently hypothesized that hyaluronic acid, which is plentiful in the rat inner medullary interstitium, could serve as a mechano-osmotic transducer, that is, that the intrinsic viscoelastic properties of hyaluronic acid could be used to transform the mechanical work of papillary peristalsis into osmotic work that could be used to concentrate urine. They proposed 3 distinct concentrating mechanisms arising from peristalsis. First, interstitial sodium activity would be reduced in the contraction phase through the immobilization of cations by their pairing with fixed negative charges on hyaluronic acid. This would result in a decreased NaCl concentration in fluid that can be expressed from the interstitium, and that relatively dilute fluid would enter the ascending vasa recta. Second, water would be absorbed in the relaxation phase from descending thin limbs as a result of decreased interstitial pressure (previously proposed by Chou et al[16] and Knepper et al[22]), and third, as a result of elastic forces exerted by the expansion of the elastic interstitial matrix arising from hyaluronic acid. If water is reabsorbed, without proportionate solute, then the descending limb tubular fluid would be relatively concentrated relative to other flows.

The hypotheses that depend on peristaltic contractions involve complex, highly coordinated cycles, with critical combinations of pressure, flow rates, permeabilities, compliances, and frequencies of peristalsis. Moreover, a determination of the adequacy of these hypotheses would appear to require a comprehensive knowledge of the physical properties of the renal inner medulla and a demonstration that the energy input from the contractions, plus any other sources of harnessed energy, is sufficient to account for the osmotic work performed. Thus, the evaluation of these hypotheses, whether by means of mathematic models or experiments, presents a daunting technical challenge.

ROLE OF THE COLLECTING DUCT

Water Transport

The collecting duct, under the influence of vasopressin, is the nephron segment that, by regulating water reabsorption, is responsible for the control of water excretion. Countercurrent multiplication in the loops of Henle generates the corticomedullary osmotic gradient necessary for water reabsorption, and countercurrent exchange in the vasa recta minimizes the dissipative effect of vascular flows. However, water excretion requires another structural component, the collecting duct system, which starts in the cortex and ends at the papillary tip. In the absence of vasopressin, all collecting duct segments are nearly water impermeable, except for the terminal IMCD, which has a moderate water permeability even in the absence of vasopressin.[51,52] Excretion of dilute urine only requires that not much water be absorbed nor much solute be secreted along the collecting duct because the fluid that leaves the thick ascending limb and enters the cortical collecting duct is dilute relative to plasma.

The entire collecting duct becomes highly water permeable in the presence of vasopressin. This occurs as follows. When blood plasma osmolality is increased, as, for example, by water deprivation, hypothalamic osmoreceptors, which can sense an increase of only 2 mOsm/kg H_2O, stimulate vasopressin secretion from the posterior pituitary gland. Vasopressin binds to V_2-receptors in the basolateral plasma

membrane of collecting duct principal cells and IMCD cells. The binding stimulates adenylyl cyclase to produce cyclic adenosine monophosphate (cAMP), which in turn activates protein kinase A, phosphorylates AQP2 at serines 256, 261, 264, and 269, inserts AQP2 water channels into the apical plasma membrane, and increases water absorption across the collecting duct[53-56] (and reviewed by Nielsen et al[57]). The major mechanism by which vasopressin acutely regulates water reabsorption is by regulated trafficking of AQP2 between subapical vesicles and the apical plasma membrane (reviewed by Nielsen et al[57]). This "membrane shuttle hypothesis," originally advanced by Wade et al,[58] proposed that water channels are stored in vesicles and inserted exocytically into the apical plasma membrane in response to vasopressin. Subsequent to the cloning of AQP2, the shuttle hypothesis was confirmed experimentally in rat inner medulla (reviewed by Nielsen et al[57]). Subsequent studies have elucidated the role of vesicle targeting proteins (synaptosome-associated protein/soluble *N*-ethylmaleimide sensitive fusion protein attachment protein receptor system [SNAP/SNARE]), several signal transduction pathways that are involved in regulating AQP2 trafficking (insertion and retrieval of AQP2), and the role of the cytoskeleton (reviewed by Nielsen et al[57]).

In the presence of vasopressin, water is reabsorbed across the collecting ducts at a sufficiently high rate for collecting duct tubular fluid to attain near-osmotic equilibrium with the hyperosmotic medullary interstitium; the reabsorbed water is returned to the systemic circulation via the ascending vasa recta. Most of the water is reabsorbed from collecting ducts in the cortex and outer medulla. Although the inner medulla has a higher osmolality than the outer medulla, its role in water reabsorption is important only when maximal water conservation is required. The IMCD reabsorbs more water during diuresis than antidiuresis, owing to the large transepithelial osmolality difference during diuresis.[59]

Urea Transport

Urea plays a special role in the urinary concentrating mechanism. Urea's importance has been appreciated since 1934 when Gamble et al[39] described "an economy of water in renal function referable to urea." Many studies have shown that maximal urine concentrating ability is decreased in protein-deprived or malnourished mammals, and urea infusion restores urine concentrating ability (reviewed by us previously[1]). Recently, a urea transporter (UT) A1/A3 knock-out mouse,[17] a UT-A2 knock-out mouse,[60] and a UT-B knock-out mouse[61-63] were each shown to have urine concentrating defects. Thus, an effect derived from urea or urea transporters must play a role in any solution to the question of how the inner medulla concentrates urine.

The initial IMCD has a low urea permeability that is unaffected by vasopressin.[51,52] In contrast, the terminal IMCD has a higher basal urea permeability than other portions of the collecting duct; either vasopressin or hypertonicity can each increase urea permeability by a factor of 4 to 6, and together they can increase urea permeability by a factor of 10 (reviewed by us previously[1]). In the 1980s, there were 3 groups that showed that vasopressin could increase passive urea permeability in isolated perfused rat IMCDs.[52,64,65] In 1987, a specific facilitated or carrier-mediated urea transport process was first proposed in rat and rabbit terminal IMCDs.[52] Subsequent physiologic studies identified the functional characteristics for a vasopressin-regulated urea transporter (reviewed by us previously[1]). To date, 2 urea transporter (UT) genes have been cloned in mammals: the UT-A (*Scl14A2*) gene encodes 6 protein and 9 complementary DNA isoforms; the UT-B (*Scl14A1*) gene encodes 2 protein isoforms[66] (reviewed by us previously[1]).

UT-A1 is expressed in the apical plasma membrane of the IMCD.[67-69] Urea transport by UT-A1 is stimulated by vasopressin when stably expressed in UT-A1 Madin-Darby kidney cells[70] and by cAMP when expressed in *Xenopus* oocytes.[71-75] UT-A3 also is expressed in the IMCD and has been detected in both the basolateral and apical plasma membranes in different studies.[76-78] Urea transport by UT-A3 is stimulated by cAMP analogs when expressed in Madin-Darby kidney cells, human embryonic kidney 293 cells, or *Xenopus* oocytes in 4 studies,[73,79-81] but not in a fifth study.[82] UT-A2, the

first urea transporter to be cloned,[83] is expressed in thin descending limbs.[68,69,84] Urea transport by UT-A2 is not stimulated by cAMP analogs when expressed in either *Xenopus* oocytes or human embryonic kidney 293 cells (reviewed by us previously[1]).

UT-B is also the Kidd blood group antigen (a minor blood group antigen in human beings) and initially was cloned from a human erythroid cell line[85] and then from rodents (reviewed by us previously[1]). UT-B protein and phloretin-inhibitable urea transport are present in descending vasa recta (reviewed by us previously[1]). Several recent studies have investigated whether UT-B transports urea only, or both water and urea.[61,86,87] Red blood cells from a UT-B/AQP1 double knock-out mouse show that UT-B can function as a water channel. However, the amount of water transported under physiologic conditions through UT-B is small (in comparison with AQP1) and is probably not physiologically significant to the urine concentrating mechanism.[62]

Rapid Regulation of Facilitated Urea Transport in the IMCD

The perfused rat IMCD has been the primary method for investigating the rapid regulation of urea transport. Although this method provides physiologically relevant functional data, it cannot determine which urea transporter isoform is responsible for a specific functional effect in rat terminal IMCDs because both UT-A1 and UT-A3 are expressed in this nephron segment. Recent studies have shown that vasopressin increases both the phosphorylation and the apical plasma membrane accumulation of both UT-A1 and UT-A3 in freshly isolated suspensions of rat IMCDs.[78,88] Vasopressin phosphorylates UT-A1 at serines 486 and 499.[89] Mutation of both serine residues eliminates vasopressin stimulation of UT-A1 apical plasma membrane accumulation and urea transport.[89] The site in UT-A3 that is phosphorylated by vasopressin has not been determined, except that neither of the 2 consensus protein kinase A sites is involved.[80] UT-A1 is linked to the SNARE machinery via snapin in rat IMCD and this interaction may be functionally important for regulating urea transport.[75]

Increasing osmolality, either by adding NaCl or mannitol, to high physiologic values as occur during antidiuresis acutely increases urea permeability in rat terminal IMCDs, even in the absence of vasopressin,[90-92] suggesting that hyperosmolality is an independent activator of urea transport. Increasing osmolality with vasopressin present has an additive stimulatory effect on urea permeability.[90-93] Hyperosmolality-stimulated urea permeability is inhibited by the urea analogue thiourea and by phloretin.[91] Kinetic studies show that hyperosmolality, similar to vasopressin, increases urea permeability by increasing V_{max} rather than K_m.[91] However, hyperosmolality stimulates urea permeability via increases in activation of protein kinase C and intracellular calcium,[94,95] whereas vasopressin stimulates urea permeability via increases in adenylyl cyclase.[96] Hyperosmolality, similar to vasopressin, increases the phosphorylation and the plasma membrane accumulation of UT-A1 and UT-A3.[78,88,97,98]

LONG-TERM REGULATION OF UREA TRANSPORTERS

Vasopressin

Administering vasopressin to Brattleboro rats (which lack vasopressin and have central diabetes insipidus) for 5 days decreases UT-A1 protein abundance in the inner medulla.[99,100] However, 12 days of vasopressin administration increases UT-A1 protein abundance.[100] This delayed increase in UT-A1 protein abundance is consistent with the time course for the increase in inner medullary urea content after vasopressin administration in Brattleboro rats.[101] Suppressing endogenous vasopressin levels by 2 weeks of water diuresis in normal rats decreases UT-A1 protein abundance.[100] Analysis of UT-A promoter I may explain this time-course because the 1.3 kb that has been cloned does not contain a cAMP response element and cAMP does not increase promoter activity.[102,103] However, a tonicity enhancer element is present in promoter I and hyperosmolality increases promoter activity.[102,103] Thus, vasopressin first directly increases the transcription of the Na-K-2Cl co-transporter NKCC2/BSC1 in the thick ascending limb; the

increase in NaCl reabsorption will increase inner medullary osmolality, which then will increase UT-A1 transcription.[104,105]

Genetic Knock-Out of Urea Transporters

Human beings with genetic loss of UT-B (Kidd antigen) are unable to concentrate their urine greater than 800 mOsm/kg H_2O, even after overnight water deprivation and exogenous vasopressin administration.[106] UT-B knock-out mice also have mildly reduced urine concentrating ability that is not improved by urea loading.[61,107] UT-A1 and UT-A3 abundances are unchanged in UT-B knock-out mice, but UT-A2 protein abundance is increased.[63] The up-regulation of UT-A2 may partially compensate for the loss of urea recycling through UT-B, thereby contributing to the mild phenotype observed in human beings lacking UT-B/Kidd antigen and in UT-B knock-out mice. The absence of UT-B also is predicted (by mathematic modeling studies) to decrease the efficiency of small solute trapping within the renal medulla, thereby decreasing urine concentrating ability and the efficiency of countercurrent exchange.[108-110] Thus, UT-B protein expression in descending vasa recta and/or red blood cells is necessary for the production of maximally concentrated urine (reviewed by us previously[1]).

UT-A1/UT-A3 knock-out mice have reduced urine concentrating ability, reduced inner medullary interstitial urea content, and lack vasopressin-stimulated or phloretin-inhibitable urea transport in their IMCDs.[17] However, when these mice are fed a low-protein diet, they are able to concentrate their urine almost as well as wild-type mice,[17] which supports the hypothesis that IMCD urea transport contributes to urine concentrating ability by preventing urea-induced osmotic diuresis.[111] Inner medullary tissue urea content was reduced markedly after water restriction, but there was no measurable difference in NaCl content between UT-A1/UT-A3 knock-out mice and wild-type mice.[17] Although this latter finding initially was interpreted as being inconsistent with the predictions of the passive mechanism,[112,113] a recent mathematic modeling analysis of these data concluded that the results found in the UT-A1/UT-A3 knock-out mice are precisely what one would predict for the passive mechanism.[5]

UREA RECYCLING

The inner medulla contains several urea recycling pathways that contribute to its high interstitial urea concentration.[111,114,115] The major urea recycling pathway is reabsorption from the terminal IMCD, mediated by UT-A1 and UT-A3, and secretion into the thin descending limb and, especially, the thin ascending limb (Fig. 4, line 1). In the inner medulla, collecting ducts and thin ascending limbs are virtually contiguous.[47,48,116,117] The urea that is secreted into the thin ascending limb is carried distally through several nephron segments having very low urea permeabilities until it reaches the urea-permeable terminal IMCD.

Two other urea recycling pathways (Fig. 4, lines 2 and 3) exist in the medulla.[115] One involves urea reabsorption from terminal IMCDs through ascending vasa recta and secretion into thin descending limbs of short-looped nephrons,[118] mediated by UT-A2,[84] or into descending vasa recta, mediated by UT-B. The other involves urea reabsorption from cortical thick ascending limbs and secretion into proximal straight tubules.[115] All 3 urea recycling pathways would limit the loss of urea from the inner medulla where it is needed to increase interstitial osmolality.[115]

In addition to urea's role in the urine concentrating mechanism, urea is the major source for excretion of nitrogenous waste and large quantities of urea need to be excreted daily. The kidney's ability to concentrate urea reduces the need to excrete water simply to excrete nitrogenous waste. A high interstitial urea concentration also serves to osmotically balance urea within the collecting duct lumen. The interstitial NaCl concentration would have to be much higher if interstitial urea were unavailable to offset the osmotic effect of luminal urea destined for excretion.[17,111]

SUMMARY

The renal medulla produces concentrated urine through the generation of an osmotic gradient extending from the corticomedullary boundary

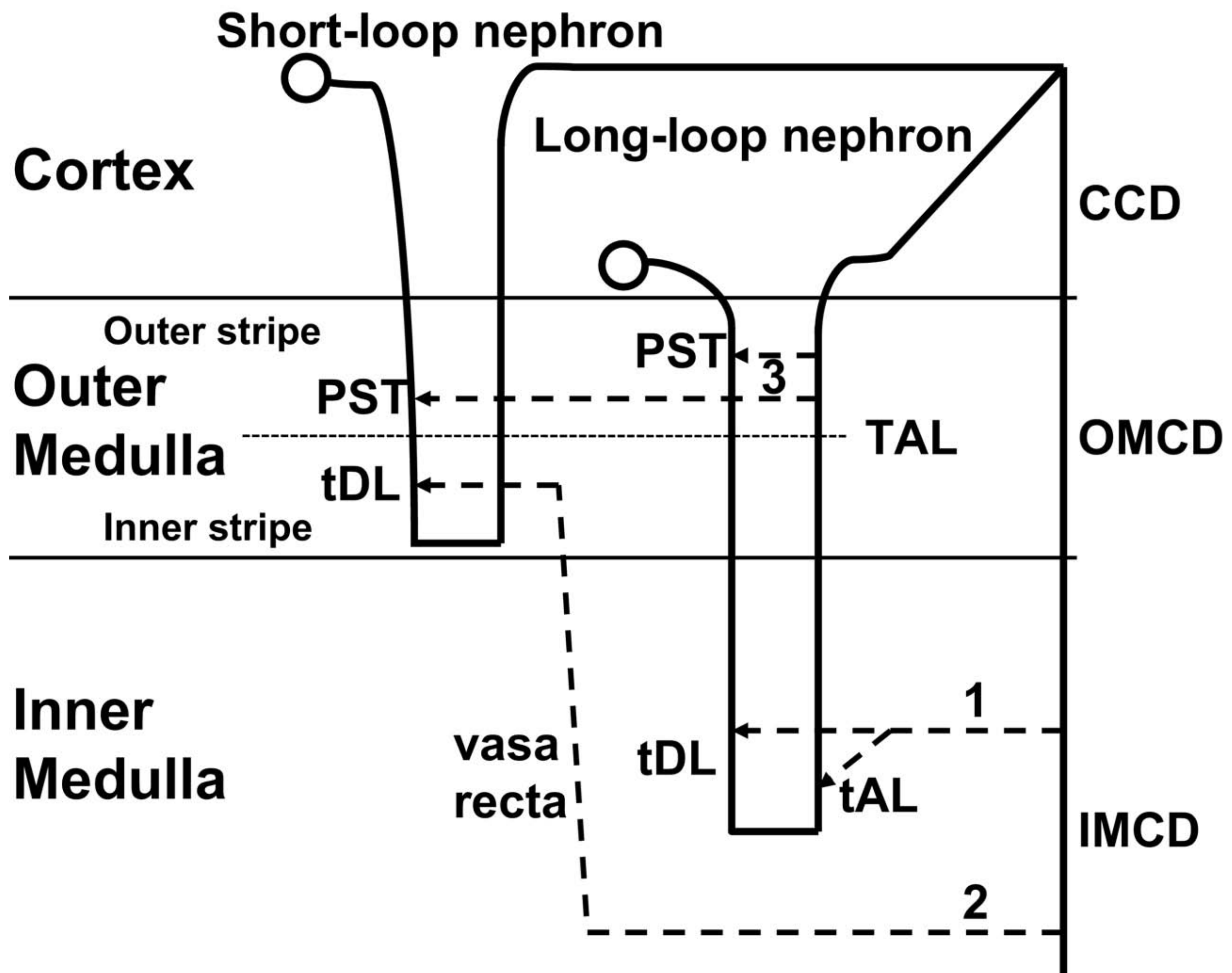

Figure 4. Urea recycling pathways in the medulla. Diagram shows a long-looped nephron (right) and a short-looped nephron (left). Dotted lines labeled 1, 2, and 3 show urea recycling pathways. PST, proximal straight tubule; tDL, thin descending limb of Henle's loop; tAL, thin ascending limb of Henle's loop; TAL, thick ascending limb of Henle's loop.

to the inner medullary tip. This gradient is generated in the outer medulla by the countercurrent multiplication of a comparatively small transepithelial difference in osmotic pressure. This small difference, called a *single effect*, arises from active NaCl reabsorption from thick ascending limbs, which dilutes ascending limb flow relative to flow in vessels and other tubules. In the inner medulla, the gradient also may be generated by the countercurrent multiplication of a single effect, but the single effect has not been identified definitively. Although the passive mechanism, proposed by Kokko and Rector[7] and by Stephenson[8] in 1972, remains the most widely accepted hypothesis for the inner medullary single effect, much of the evidence from perfused tubule and micropuncture studies is either inconclusive or at variance with the passive mechanism. Moreover, the passive mechanism has not been supported when measured transepithelial transport parameters are used in mathematic simulations.

Nevertheless, there have been important recent advances in our understanding of key components of the urine concentrating mechanism. In particular, the identification and localization of key transport proteins for water, urea, and sodium, the elucidation of the role and regulation of osmoprotective osmolytes, better resolution of the anatomic relationships in the medulla, and improvements in mathematic modeling of the urine concentrating mechanism. Continued experimental investigation of transepithelial transport and its regula-

tion, both in normal animals and in knock-out mice, and incorporation of the resulting information into mathematic simulations, may help to elucidate more fully the inner medullary urine concentrating mechanism.

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