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REVIEW ARTICLE

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Phylogenetic, ontogenetic, and pathological aspects of the urine-concentrating mechanism

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Abstract
The urine-concentrating mechanism is one of the most fundamental functions of avian and mammalian kidneys. This particular function of the kidneys developed as a system to accumulate NaCl in birds and as a system to accumulate NaCl and urea in mammals. Based on phylogenetic evidence, the mammalian urine-concentrating mechanism may have evolved as a modification of the renal medulla's NaCl accumulating system that is observed in birds. This qualitative conversion of the urine-concentrating mechanism in the mammalian inner medulla of the kidneys may occur during the neonatal period. Human kidneys have several suboptimal features caused by the neonatal conversion of the urine-concentrating mechanism. The urine-concentrating mechanism is composed of various functional molecules, including water channels, solute transporters, and vasopressin receptors. Abnormalities in water channels aquaporin (AQP)1 and AQP2, as well as in the vasopressin receptor V2R, are known to cause nephrogenic diabetes insipidus. An analysis of the pathological mechanism involved in nephrogenic diabetes insipidus suggests that molecular chaperones may improve the intracellular trafficking of AQP2 and V2R, and, in the near future, such chaperones may become a new clinical tool for treating nephrogenic diabetes insipidus.

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Introduction

The urine-concentrating mechanism performs one of the most essential functions in water and electrolyte metabolism during childhood development. In the fetal period, the urine-diluting ability is mature. However, the urine-concentrating mechanism undergoes profound development during the neonatal period. During the weaning period, the ability to concentrate urine reaches maturity, and it is at this time that solid food, which is a more efficient source of energy, is introduced.

In the neonate, because the ratio of body surface area to mass is greater than in the adult, insensible water loss, which can occur during various pathological conditions, such as fever, vomiting, and diarrhea, is more likely to cause dehydration. Therefore, a disruption of the urine-concentrating mechanism in the neonatal period leads to an immediate threat to life.

In this article, the basic ontogeny and phylogeny of the urine-concentrating mechanism, the roles of the various transport systems in the renal tubules, and their importance during the development of the urine-concentrating mechanism will be discussed.

Outline of the urine-concentrating mechanism

Relationship to renal tubular function

The urine filtered in the glomeruli is transferred to the proximal tubule and then reaches the renal medulla. From the proximal tubule the urine flows through each segment of Henle's loop, the distal convoluted tubule, the connecting tubule, and the collecting ducts. Each nephron segment

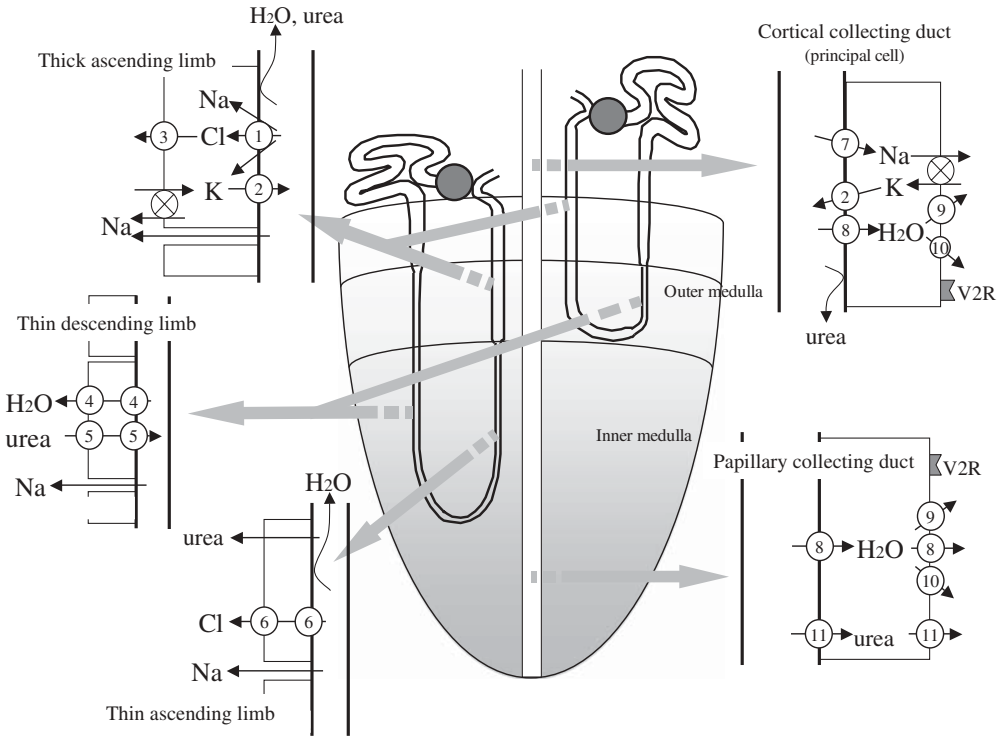

Fig. 1. Mechanisms of NaCl, urea, and water transport in each medullary nephron segment. The major mechanisms of NaCl, urea, and water transport are depicted. Long-looped and short-looped nephrons are shown on the *left side* and *right side* of the collecting duct, respectively. Major transporters involved in the urine-concentrating mechanism are indicated as follows: *1*, Na^+-K^+-2Cl^- contransporter NKCC2; *2*, K^+ channel ROMK; *3*, Cl^- channel CLC-Kb; *4*, water channel AQP1; *5*, urea transporter UT-A2; *6*, Cl^- channel CLC-Ka; *7*, epithelial Na^+ channel ENaC; *8*, vasopressin-sensitive water channel AQP2; *9*, water channel AQP3; *10*, water channel AQP4; *11*, vasopressin-sensitive urea transporter UT-A1; *V2R*, vasopressin V2 receptor

has specific transport properties for water and electrolytes, and the complex structure of the tubules and blood vessels contributes to the construction of an effective urine-concentrating mechanism in the kidney. Figure 1 shows a model of the water and electrolyte metabolism that occurs in each nephron segment of the kidney.

Reabsorption of urine in the proximal tubule is almost completely an isosmotic process, in which the osmolar gradient between the urine and blood is minimal. The data of Barfuss and Schafer[1] and the analyses of AQP1-knockout mice[2,3] show that water reabsorption in the proximal tubule is supported by a slight transepithelial osmotic gradient generated by NaCl and bicarbonate reabsorption in this segment. Large changes in urine osmolarity occur in the renal medulla. It is well known that the transport properties of the descending limbs of Henle's loop in the short-looped nephron differ from those of the upper and lower portions of the long-looped nephron,[4–8] even though all of these segments possess high amounts of AQP1 in both the luminal and basolateral membranes. In the medulla, the whole thin descending limb plays an important role in reabsorbing water from the urine, thereby contributing directly to urine concentration. On the other hand, the ascending limbs of Henle's loop are called "diluting segments", because they have special membrane transport properties that reabsorb NaCl without reabsorbing any water.[9,10] The thin ascending limb, which is located only in the long-looped nephron, has very high permeability to NaCl that is enhanced by paracellular passive Na permeability and transcellular passive Cl permeability via the Cl^{-1} channel CLC-Ka, located in both the luminal and the basolateral cell membranes.[11–13] In the thick ascending limbs, NaCl is actively reabsorbed via the luminal Na^+-K^+-2Cl^- cotransporter NKCC2. Both the distal convoluted and the connecting tubules are impermeable to water, whether or not vasopressin is present.

The transport properties of the collecting duct system are controlled to a great extent by vasopressin and aldosterone. In the absence of vasopressin, the water permeability of the collecting duct system is almost negligible, while under antidiuretic conditions, enormous AQP2 water channels are inserted into the luminal cell membranes of the principal cells. It is also known that the urea permeability of the papillary collecting duct is also increased under antidiuretic conditions.[14] The mechanisms of the changes in water and urea permeability in response to vasopressin differ in various respects.[15]

The urine-concentrating mechanism and its regulation

Vasopressin, the most powerful regulator of the urine-concentrating mechanism, is a peptide hormone, composed of nine amino acids, that is produced in the brain's supraoptic nuclei, as well as in the hypothalamo-paraventricular nuclei. Neurophysin2 cleaves vasopressin from propressophysin, which is a large precursor of oxytocin found in the secretory granules; vasopressin is then transported to the posterior lobe of the pituitary gland.[16] The secretion of vasopressin is regulated by the osmoreceptor in the frontolateral region of the third ventricle, where there is no blood-brain barrier. There is no definite consensus regarding the nature of the brain's osmoreceptor. Thus far, AQP4[17] and the vanilloid receptor-related osmoacceptor channel VR-OAC[18] have been considered to be candidate osmoreceptors.

Thus far, three receptors, V1aR, V1bR, and V2R, have been reported as being vasopressin receptors, with V2R

being the most important receptor that is intimately related to the urine-concentrating mechanism. Stimulation of V2R by vasopressin facilitates the production of cyclic AMP via the activation of Gs protein and adenylate cyclase, which are coupled with the receptor. The subsequent activation of protein kinase A leads to the facilitation of the urine-concentrating mechanism. Thus far, stimulation of V2R in animals has been shown to facilitate NaCl reabsorption in the thin[19] and thick ascending limbs, accelerate NaCl reabsorption in the cortical collecting duct,[20] facilitate water permeability in the cortical and medullary collecting ducts, and stimulate urea reabsorption in the inner medullary collecting duct.[21–23] In humans, it has been demonstrated that vasopressin facilitates water[24] and urea[14] transport in the collecting duct system. Stimulation of NaCl reabsorption in the thick ascending limb of Henle's loop does not take place in humans.[25] There is no information regarding vasopressin's role in NaCl transport in the thin ascending limb in humans.

Ontogeny and development of the urine-concentrating mechanism

Ontogeny of the urine-concentrating mechanism

In the fetal period, three independent kidneys emerge sequentially. In order of emergence, they are called the pronephros, mesonephros, and metanephros. The metanephros forms the final mature kidney, and develops by the fourth fetal month. In the metanephros, contact between the mesenchymal blastema and the ureteric bud initiates development. These structures form the glomeruli and the tubules by facilitating each other's differentiation. The *PAX-2* gene, which is expressed in the ureteric bud, regulates the branching and elongation of the renal tubules by interacting with the secretion of glial cell line-derived neurotrophic factor (GDNF) from the mesenchymal cells.[26] Recent studies have found that various molecular regulatory processes, including the transforming growth factor (TGF)-β superfamily, control renal development.[26–29]

Production of urine starts by the fifth fetal month. By the end of fetal life, urine is produced at a rate of 50 ml/h. Fetal urine is slightly hypotonic compared to body fluid, while it becomes hypertonic soon after birth.[30,31]

The water permeability of the collecting duct system is already sensitive to vasopressin at birth.[32] In rats, the entire Henle's loop is known to be impermeable to water,[33] while in human fetuses, the descending thin limb already possesses water permeability.[34]

Recently, nephrologists have become interested in the fact that the neonatal thin ascending limb of Henle's loop has a thick morphological appearance.[35,36] Our recent studies of rat kidneys found that in neonatal rats, the entire ascending limb of Henle's loop, including the thin and thick ascending limbs, could actively reabsorb NaCl, which, in the mature kidney, occurs only in the thick ascending limb.[33] We have also demonstrated that, in the inner medulla of neonatal rat kidneys, the distribution of NKCC2[37] in the luminal membrane and CLC-K1[38,39] is reversed compared with that in the mature animal.[33] On day 0, the rat inner medulla expresses antigenicity to NKCC2, which is expressed only in the outer medulla of the mature kidneys. As well, CLC-K1, which is present in the mature inner medulla, is not expressed in the neonatal inner medulla. It has also been demonstrated that the vasopressin-sensitive urea transport system is not functional in the neonatal rat inner medullary collecting duct.[33] In the inner medullary collecting duct, permeability to urea and sensitivity to vasopressin have been shown to emerge during the weaning period.

These characteristics of neonatal renal medullary organization lead us to the conclusion that the urine-concentrating mechanism dependent on urea is not mature in neonates. This observation implies that the neonatal urine-concentrating mechanism utilizes only NaCl for generating the medullary osmotic gradients essential for water reabsorption from the urine in the neonatal renal tubules. Thus, during infancy, rapid developmental changes occur in the urine-concentrating mechanism to convert it from simple NaCl accumulation to the accumulation of both NaCl and urea. This understanding of the development of the urine-concentrating mechanism may help us explain the low urine-concentrating ability during the neonatal period.

Phylogeny of the urine-concentrating mechanism

It is interesting to understand how the kidneys evolved to handle both water and electrolytes. In 1974, Valtin depicted the progression of evolutionary changes of the renal glomerular and tubular structures. The fundamental differences in the handling of water and electrolytes in various animals are summarized in Fig. 2. Fish migrated from sea water to fresh water and subsequently developed large glomeruli and the diluting tubule segment, which at first secreted massive amounts of body fluid and recovered essential components such as electrolytes and metabolites. The glomeruli and renal tubules formed a functional combination that allowed large amounts of water to be eliminated in the urine. In contrast to the threat of overhydration that freshwater fish faced, reptiles evolving from amphibians encountered the threat of dehydration due to the dry conditions found on land. Reptiles, which did not develop a urine-concentrating mechanism, may have adapted by minimizing glomerular filtration, thereby minimizing the amount of urine that would be produced. The history of vertebrate evolution does not suggest that birds and mammals evolved from the same species,[40] but the urine-concentrating mechanism may have evolved during the process of vertebrates adapting and achieving homeothermy. The fact that only birds and mammals can concentrate urine, have Henle's loop, and are homeothermic suggests an interesting evolutionary process.

In mammals, a urine-concentrating mechanism that is more dependent on urea than NaCl is the major tool to deal with a dry environment. In birds, both the regulation of glomerular filtration and a urine-concentrating mechanism

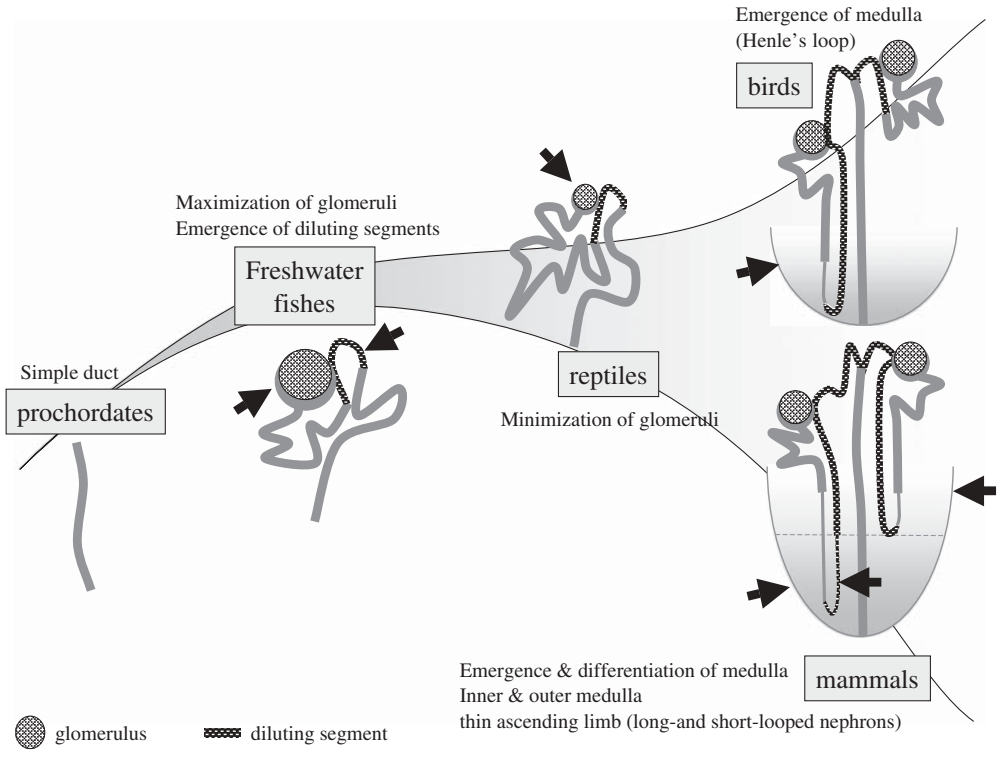

Fig. 2. Differences in water and electrolyte handling among selected animals. The major evolutionary differences of nephron structures in selected animals are depicted. In freshwater fish, the size of the glomerulus is maximized to increase the glomerular filtration volume for producing massive amounts of hypotonic urine. The emergence of the diluting nephron segment is also characteristic. In reptiles, the size of the glomerulus is minimized to preserve water in the dry land atmosphere. Henle's loop emerges in both birds and mammals, while mammalian kidneys possess short-looped and long-looped nephrons. The emergence of the thin ascending limb of Henle's loop indicates the differentiation of the inner medulla in mammalian kidneys

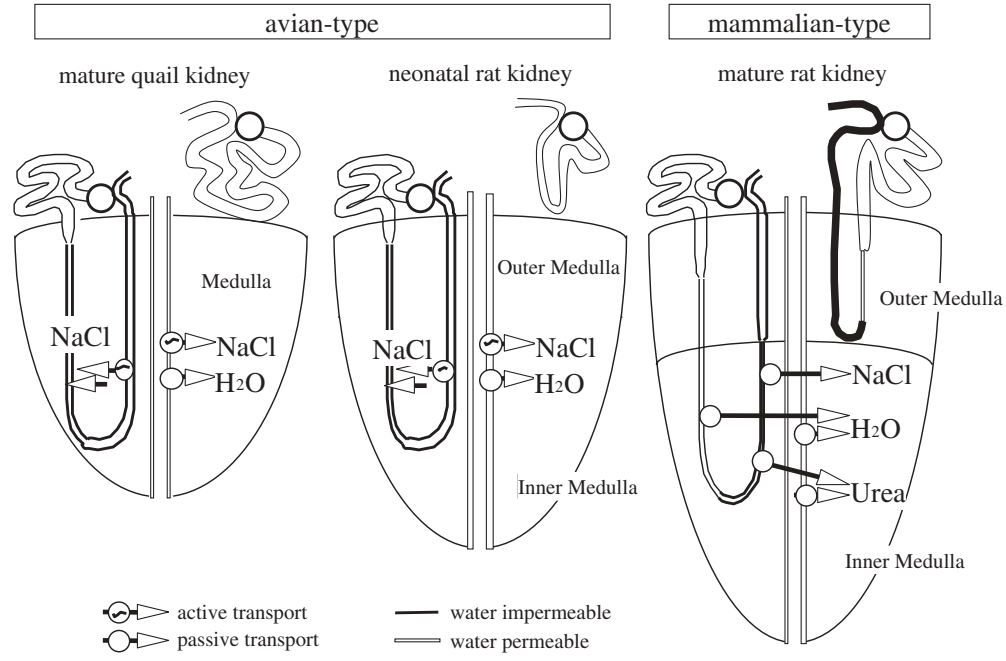

Fig. 3. Comparison of the transport properties of renal medullary tubules in mature quails, neonatal rats, and mature rats. The organization of the urine-concentrating mechanism in the renal medullae of mature quails, neonatal rats, and mature rats is depicted. Mature quail and neonatal rat kidneys both possess Henle's loops, which are entirely impermeable to water, and the ascending limbs actively reabsorb NaCl all along their lengths. In mature rat kidneys, the descending limb of Henle's loop is highly permeable to water, the thin ascending limb is highly permeable to NaCl, and urea permeability is present in the inner medullary collecting ducts and thin limbs

that is dependent on NaCl play important roles in water and electrolyte homeostasis. From the phylogenetic perspective, the urine-concentrating mechanism is unique in that it is only present in the metanephros, which emerges only in birds and mammals. Urine-concentrating ability develops after birth and matures during the weaning period. In this sense, maternal feeding appears to supplement the neonate's immature urine-concentrating ability.

Changes in the renal medullary tubular transport properties of mature quails,[41–44] neonatal rats, and mature rats are compared in Fig. 3.[33] The figure indicates that the functional organization of the medullary tubules in neonatal rats is very similar to that in mature quails, in that the entire Henle's loop is impermeable to water, and the entire ascending limb actively reabsorbs NaCl. The comparison also clearly shows that, during the process of maturation in rats,

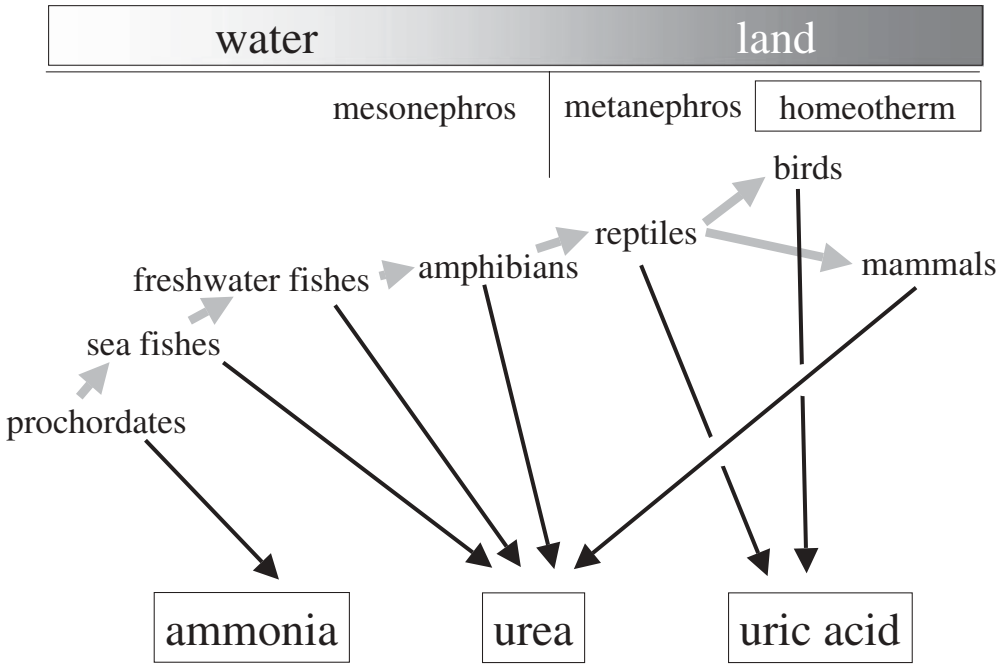

Fig. 4. The evolution of vertebrates and their nitrogen metabolism. The evolutionary changes in nitrogen metabolites in vertebrates are depicted. It is noteworthy that mammals, which evolved from reptiles, started to utilize urea as the major nitrogen waste metabolite as do fish and amphibians, although when reptiles evolved from amphibians they did not use urea

the inner medullary tubular function is replaced by transport properties that use urea as the urine-concentrating mechanism.

Given that birds and mammals are thought to have independent origins with respect to vertebrate evolution, the comparison suggests that the mammalian urine-concentrating mechanism evolved in two steps: first, simple NaCl accumulation, and then complex NaCl and urea accumulation.

It is quite a challenge to trace the evolution of nitrogen metabolism and compare it with the evolution of the urine-concentrating mechanism (see Fig. 4 for schema). While the most primitive prochordates utilized ammonia to excrete nitrogen waste, fish began to use urea, which is a relatively simple nitrogen metabolite. Some fish, including sharks, use urea for adjusting their buoyancy in sea water. There is no proof that urea is used to maintain water balance in fish. Amphibians also utilize urea to excrete nitrogen waste. Interestingly, the urea permeability of the toad bladder is sensitive to vasotocin, which indicates that urea is already used to maintain water balance in this species. However, reptiles, the first pure land vertebrates, do not use urea for nitrogen excretion because the urea molecule is highly water-soluble. Reptiles must tolerate a very dry external environment, which may have favored the evolution of hard-shell eggs in the fetal period; this, in turn, may have hindered the production of urea in the eggs. Thus, these animals began to produce uric acid, which does not dissolve easily and which forms uric acid crystals for excretion from body fluids. As a matter of fact, the eggs of reptiles possess an allantoic sac, in which, during the fetal period, nitrogen waste is accumulated as uric acid. Uric acid is also used for excreting nitrogen waste. However, in mammals, urea appears to have been revived as the major vehicle for excreting nitrogen waste. Thus, taking into account phylogenetic consistency, it seems that the mammalian urine-concentrating mechanism may be related to a switch from excreting nitrogen waste using uric acid to using urea. Because mammals have a very high metabolic rate, they need to have a very efficient urine-concentrating mechanism, which may have favored the reversion from uric acid to urea as the mechanism of nitrogen waste excretion.

In the field of biology, phylogenetic hypotheses are not always relevant, and one must be very cautious in interpreting evolutionary evidence. However, consideration of phylogenetic aspects can be a very useful tool for developing a research strategy. Thus, thoughtful consideration of the phylogenetic aspects of the vertebrate urine-concentrating mechanism could further our research in this area.

Functional molecules of the urine-concentrating mechanism

Disruption of the urine-concentrating mechanism causes diabetes insipidus. Nephrogenic diabetes insipidus is a serious disorder that may sometimes cause death due to dehydration, as a result of the excretion of massive amounts of hypotonic urine. As this disease results from the kidneys' insensitivity to vasopressin, treatment is more complicated than that for central diabetes insipidus.

To provide a better understanding of the nature of the urine-concentrating mechanism, the major functional molecules are summarized below.

Vasopressin receptors

The V2 receptor of vasopressin is one of the most important molecules in the urine-concentrating mechanism. Approximately 95% of nephrogenic diabetes insipidus is caused by

abnormal function of the V2 receptor. Although congenital nephrogenic diabetes insipidus is a rare disorder, more than 130 different mutations of the V2 receptor gene have been reported to cause nephrogenic diabetes insipidus. There are at least three vasopressin receptors: V1aR,V1bR, and V2R. All of them are membrane proteins that have seven transmembrane domains and activate G proteins to stimulate signal transduction within cells.

Water channels

Since CHIP28[45] was first identified as a water channel in red blood cells and was cloned as aquaporin-1(AQP1), more than ten aquaporins, e.g., AqpZ, GlpF, and AQP0 to AQP10, have been discovered. Peter Agre[46] was awarded the Nobel Prize in 2003 for discovering AQP1. The aquaporins are classified into two groups. The aquaporins, including AqpZ,[47] AQP0,[48–50] AQP1,[45] AQP2,[51] AQP4,[17] AQP5,[52] AQP6,[53] and AQP8,[54] are only permeable to water, while the aquaglyceroporins, such as GlpF,[55,56] AQP3,[57–59] AQP7,[60] AQP9,[61] and AQP10,[62] are permeable to both water and glycerol. AQP11 was discovered in 2004;[63] this aquaporin is called superaquaporin due to its poor conservation of asparagine-proline-alanine (NPA) boxes, which are important for the formation of the water-permeating pore. The disruption of AQP11 has been reported to cause urological disorders, including polycystic kidney disease.[63,64] Recently, AQP12 has been identified in pancreatic acinar cells.[65] More aquaporins may be discovered in the future.

The various aquaporins are located in the luminal, basolateral, and intracellular membrane components of the renal tubules. Among them, the most important aquaporin related to diabetes insipidus is aquaporin-2 (AQP2), which is located in the principal cells of the collecting duct system.

The aquaporin-2 gene is located on chromosome 12 (12q13) and is composed of four exons and three introns.[66] AQP2 is a membrane protein that has six transmembrane domains, and is composed of 271 amino acids.

The disruption of AQP1 causes nephrogenic diabetes insipidus in a knockout mouse model. In humans, AQP1 deficiency may impair urine-concentrating ability.

The disruption of AQP3 and AQP4 also causes nephrogenic diabetes insipidus in knockout animal models, though the disease caused by the disruption of AQP3 and AQP4 is unknown in humans. Many aquaporins play critical roles in maintaining the urine-concentrating mechanism.

Urea transporters

In mammals, urea transporters are thought to play a critical role in the concentrating of urine. Due to its hydrophilic properties, urea is fundamentally impermeable to the lipid bilayers of the cell membranes. Urea is dissolved in water at more than 5 moles/l, and may require specific membrane transporters for transmembranous transport in the kidney.

The first urea transporter to be identified was the vasopressin-sensitive urea transporter UT2, from cDNA libraries of the rabbit renal medulla.[67] Urea transporters can be classified into two groups, UT-A and UT-B. UT-B is known as the Kidd blood antigen.[68,69] The human *Slc14a1* gene, which encodes UT-B, is on chromosome 18 (18q12). The *A1c14a2* gene, which encodes UT-A, is located at a locus close to the *Slc14a1* gene.[70]

In the kidneys, UT-B1 is located in the descending vasa recta (DVR). Of the UT-A urea transporters, UT-A1 and UT-A3 are located in the inner medullary collecting duct, and UT-A2 is located in the thin descending limb. In addition to these urea transporters, UT-A1b, UT-A2b, UT-A3b, UT-A4, and UT-A5 have been identified, but their localization and function in the kidneys is not clear. In 2004, Fenton et al.[71] disrupted UT-A1 and UT-A3 by deleting the *3 kb* gene, including exon 10, in a mouse model, and found that nephrogenic diabetes insipidus was caused. Interestingly, the accumulation of NaCl in the renal medulla was not impaired, while the accumulation of urea was strongly reduced.

To date, there have been no reports that a molecular disturbance of urea transporters causes nephrogenic diabetes insipidus in humans.

Chloride channels

Chloride channels are known to be related to nephrogenic diabetes insipidus. In 1990, a chloride channel named CLC-0 was first identified in *Torpedo marmorata*, and using a homology cloning strategy, this was followed by the discovery of more types.[38,72–80] In the kidneys, almost all of the chloride channels, except for CLC-1, have been identified.

CLC-K1 (CLC-Ka in human) is the first chloride channel that was identified by a polymerase chain reaction (PCR) cloning strategy. CLC-K1 is located in the thin ascending limb of Henle's loop. Both *CLCNKA*, the gene for CLC-Ka, and *CLCNKB*, the gene for CLC-Kb, are located on chromosome 1 (1p36),[81] and have high homology. CLC-Ka is present in both the luminal and basolateral membranes of the thin ascending limb of Henle's loop. CLC-K1 facilitates the dilution of hyperosmolar urine by passively reabsorbing chloride without the transepithelial movement of water. In 1999, disruption of CLC-K1 in mice was shown to cause nephrogenic diabetes insipidus, indicating that the inner medullary component of Henle's loop plays an important role in the urine-concentrating mechanism. Thus far, there have been no reports of an isolated abnormality of human CLC-Ka causing nephrogenic diabetes insipidus. However, a mutation of barttin,[82] the β subunit of both CLC-Ka and Kb, has been reported to cause Bartter syndrome with sensory deafness.[82,83]

Clinical features and treatment of nephrogenic diabetes insipidus

Clinical features

Nephrogenic diabetes inspidus is classified into two types – congenital and acquired, as shown in Table 1. The acquired

Table 1. Causes of nephrogenic diabetes insipidus

Congenital	
X-linked	AVPR2
Autosomal recessive	AQP2
Autosomal dominant	AQP2
Other	
Acquired	
Drug-induced (lithium, demeclocycline, amphotericin, methoxyflurane)	
Hypercalcemia, hypokalemia	
Obstruction of urinary tract	
Other	

causes include drugs, such as lithium and amphotericin; hypercalcemia; hypokalemia; and obstruction of the urinary tract.[84]

Congenital nephrogenic diabetes insipidus is diagnosed mainly in childhood; polydipsia and polyuria are the major clinical manifestations. In the neonatal and infantile periods, these symptoms can easily lead to severe dehydration, with fever and convulsions. Congenital nephrogenic diabetes insipidus can be caused by an X-linked recessive, an autosomal recessive, or an autosomal dominant trait.[84]

X-linked nephrogenic diabetes insipidus is caused by the disruption of the vasopressin V2 receptor V2R on the X chromosome (Xp28).[85,86] Thus far, more than 155 types of mutations in 239 families have been found; the locations of the mutation are distributed over the gene's entire territory.[87] The types of mutations include nonsense, frameshift, and missense. The most important pathological mechanism of these mutations is disturbance of the mutated protein's translocation. The receptor peptides formed in the endoplasmic reticulum (ER) are transferred to the Golgi complex. Mutation of the receptor gene leads to a disturbance of protein folding and, thereby, hinders the transfer of the protein. The mutated protein that is left behind in the ER is catalyzed in the endosome. This pathological mechanism is the one most commonly seen in X-linked nephrogenic diabetes insipidus. In addition to a disturbance of protein translocation, abnormalities of vasopressin receptor-binding, G-protein activation, and cAMP production have also been reported.

Autosomal recessive and dominant forms of nephrogenic diabetes insipidus are mainly caused by an *AQP2* mutation. Thus far, more than 26 different mutations in 25 families have been reported. The *AQP2* gene was identified in rats[51] in 1993 and in humans[66] in 1994. In 1993, mutation of the *AQP2* gene was shown to cause autosomal recessive nephrogenic diabetes insipidus.[51] Later reports elucidated that mutation of the *AQP2* gene could also cause autosomal dominant nephrogenic diabetes insipidus. It is now known that almost 25% of the *AQP2* mutations manifest as autosomal dominant nephrogenic diabetes insipidus. It is of great interest that an abnormality of the *AQP2* gene mainly leads to a disturbance in the translocation of the AQP2 peptide, which is similar to that observed in cases of V2R mutations.[87,88] It is also of interest that most of the mutations that occur with the autosomal dominant mutation of the *AQP2* gene are located near the C-terminal of AQP2.

Thus far, all identified molecular abnormalities that result in nephrogenic diabetes insipidus have involved only V2R, AQP2, and AQP1. Further research may identify the involvement of other transporters, including the remaining AQPs, urea transporters, and chloride channels, in the development of nephrogenic diabetes insipidus.

Treatment of nephrogenic diabetes insipidus

Acquired nephrogenic diabetes insipidus is treated by elimination of the cause. Should the removal of the cause not affect the clinical course of the disease, symptomatic treatment is given. In cases caused by urinary tract obstruction, surgery is the treatment of first choice.

The fundamental approach to the treatment of nephrogenic diabetes insipidus has not been well established. In general, restriction of salt intake and treatment with thiazide diuretics are used to reduce the massive urine volume. Thiazide diuretics are thought to reduce urine volume by decreasing NaCl reabsorption in the distal convoluted tubule via the specific inhibition of the luminal NaCl cotransporter NCCT; this causes Na depletion, reduces glomerular filtration, and facilitates reabsorption of water in the proximal tubule. Thiazides can be expected to reduce urine volume by 30% to 50%. Pattaragarn and Alon[89] reported a 1-month-old boy treated successfully with a combination of hydrochlorothiazide and a cyclooxygenase-2 inhibitor, rofecoxib, without any drug-related side effects, such as gastrointestinal bleeding. Such combination therapies need to be studied so as to further improve the prognosis of the disease.

Currently, treatments using molecular chaperones for the mutated proteins are attracting attention. Deen et al.,[90] in 1994, and Mulders et al.,[91] in 1997, reported cases of autosomal recessive nephrogenic diabetes insipidus related to the abnormal intracellular trafficking of AQP2 molecules. They concluded that the AQP2 molecules had the normal properties of AQP2, but that their trafficking to the luminal cell membrane was disturbed.[91,92] In 1998, Tamarappoo and Verkman[93] showed that abnormal AQP2 function, due to a disturbance in the transfer of the molecules from the intracellular ER, was corrected by adding chemical chaperones, such as glycerol, trimethylamine oxide (TMAO), and dimethylsulfoxide (DMSO), in expression experiments in Chinese hamster ovary (CHO)-K1 cells, in Madin-Darby canine kidney (MDCK) cells, and in a water permeability test in oocytes. In 2003, Tan et al.[94] reported that, in cases of V2R mutations, such as L292P, in which the process after glycosylation was disturbed, the membrane-permeable V2 receptor antagonist SR121463B strengthened the stereoscopic stability of the V2 receptor, thereby facilitating the transfer of the receptor molecules to emerge on the cell surface. In the near future, further studies will focus on the clinical applicability of these findings.

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