Key Roles of Renal Aquaporins in Water Balance and Water-Balance Disorders

Søren Nielsen, Tae-Hwan Kwon, Jørgen Frøkiær, and Mark A. Knepper

The discovery of aquaporins by Agre and co-workers provided an answer to the long-standing biophysical question of how water can pass cell membranes. The identification and characterization of several aquaporins expressed in the kidney has allowed detailed insight, at the molecular level, into the fundamental physiology and pathophysiology of renal water metabolism.

Renal regulation of body water balance involves reabsorption of water in the proximal nephron and vasopressin-regulated water reabsorption in the collecting duct. At least six aquaporins (AQP1, -2, -3, -4, -6, and -7) are presently known to be expressed in the kidney (Table 1). AQP1 is highly abundant in the proximal tubule and descending thin limb, and several studies have now underscored its important role in constitutive water reabsorption in these segments. AQP1 is absent in other tubule segments. At least three aquaporins are known to be expressed in the kidney collecting duct, and they participate in...
vasopressin-regulated water reabsorption. AQP2 is the apical water channel of collecting duct principal cells and is the chief target for short-term regulation of collecting duct water permeability by vasopressin (9). In addition, collecting duct water reabsorption is regulated on a long-term adaptational basis, and several studies have provided strong support that long-term regulation of AQP2 expression is a key factor in this. Thus there is a long-term regulation of the total abundance of AQP2 in collecting duct cells, which can then enter the short-term regulated trafficking to regulated collecting duct water reabsorption. AQP3 and AQP4 are both expressed in the basolateral plasma membranes of collecting duct principal cells and represent potential exit pathways for water reabsorbed via AQP2. Recent studies have underscored the role of aquaporins, and especially AQP2, in short-term and long-term regulation of body water balance. Moreover, a series of studies has also implicated important roles of AQP2 in several inherited and acquired water balance disorders. AQP6 and AQP7 are also expressed in the kidney, but their specific location is currently unknown.

**Aquaporin structure**

Aquaporins are water channels allowing passive flux of water across the membrane. The prototypical aquaporin, AQP1 or CHIP28, was discovered by Agre and colleagues (for recent review, see Ref. 1). According to phylogenetic properties and their specificity for water and other solutes, aquaporins have been divided into two principal groups: the “orthodox set” (AQP5), which selectively transport water, and the “cocktail set” (aquaglyceroporins), which also carry other small molecules such as glycerol (1). Although the structural characteristics responsible for the transport specificity and selectivity are poorly understood, the three-dimensional structure and oligomeric organization of AQP1 is emerging. Structurally, aquaporins have six membrane-spanning domains, intracellular amino and carboxyl terminals, and internal tandem repeats that are believed to be a consequence of an ancient gene duplication (1). Of the five connecting loops in AQP1, the B and E loops presumably dip into the lipid bilayer and form “hemichannels” that connect between the leaflets to form a single pathway within a symmetrical structure that resembles an hourglass. The three-dimensional structure of AQP1 has recently been determined at 6-Å resolution by cryoelectron microscopy (15). AQP1 and possibly several other aquaporins, such as AQP2 and AQP3, form tetramers in the membrane. However, AQP4 in glial cells and the basolateral plasma membrane of collecting duct principal cells assembles rather uniquely in a multimeric structure that, visualized by freeze-fracture, appears as intramembrane particle square arrays.

**Renal aquaporins**

Absorption of water in the renal tubule depends on the driving force for water reabsorption and osmotic equilibration of water across the tubular epithelium (9). The driving force is established, in part, via active NaCl transport, and generation of a hypertonic medullary interstitium results as a consequence of countercurrent multiplication. This requires active transport and low water permeability in some tubule segments and high water permeability (constitutive or regulated) in other segments. It is now clear that osmotic water transport across the tubule epithelium is chiefly dependent on aquaporin water channels.

The critical role of AQP1 in urinary concentration was recently highlighted in studies using transgenic mice with
knockout of the AQP1 gene. These mice were polyuric and had a reduced urinary concentrating capacity. Moreover, isolated perfused proximal tubules and descending thin limbs had an 80 and 90% reduction in osmotic water permeability, respectively, illustrating an important role of AQP1 in water transport across these tubule segments (14). Moreover, these studies also emphasized the important role of transcellular rather than paracellular water transport in these tubule segments.

AQP2 (8) is expressed in principal cells of the cortical, outer, and inner medullary collecting ducts (Fig. 1 and Table 1) and is abundant both in the apical plasma membrane and subapical vesicles. AQP2 is the primary target for vasopressin regulation of collecting duct water permeability (9). This conclusion was established in studies showing a direct correlation between AQP2 expression and collecting duct water permeability in rats (6) and in studies demonstrating that humans with mutations in the AQP2 gene (5) or rats with 95% reduction in AQP2 expression (10) have profound nephrogenic diabetes insipidus. As described below, body water balance is regulated in part by short-term and long-term regulation of the collecting duct water permeability. The acute vasopressin-induced increase in collecting duct water reabsorption has been shown to involve vasopressin-regulated trafficking of AQP2 between intracellular vesicles and the apical plasma membrane. Long-term regulation of AQP2 involves mechanisms that alter the total abundance of AQP2 protein, thereby modulating the acute response by changing the number of water channels in the cell that can be recruited for vasopressin-regulated trafficking. The abundance of AQP3 also appears to be regulated by factors related to water intake. Thus the short-term and long-term mechanisms act together in a concerted fashion to regulate body water balance (Fig. 2; see below).

Regulation of AQP2 trafficking by vasopressin

As illustrated in Fig. 3, a marked redistribution of AQP2 from intracellular vesicles to the apical plasma membrane occurs in response to vasopressin stimulation. This was demonstrated in isolated perfused inner medullary collecting ducts that were fixed for immunochemistry. Conversely, removal of vasopressin induced a decrease in AQP2 in the apical plasma membrane in association with the reappearance of AQP2 in intracellular vesicles. These changes in the subcellular distribution of AQP2 were paralleled by changes in water permeability of the same tubules. This provided direct

<table>
<thead>
<tr>
<th>Aquaporins</th>
<th>Number of Amino Acids</th>
<th>Kidney Localization</th>
<th>Extrarenal Localization</th>
<th>Subcellular Distribution</th>
<th>Regulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>AQP1</td>
<td>Human</td>
<td>269</td>
<td>Proximal tubules</td>
<td>Multiple organs</td>
<td>APM/BLM</td>
</tr>
<tr>
<td>AQP2</td>
<td>Rat</td>
<td>271</td>
<td>Collecting duct principal cells</td>
<td>Testis</td>
<td>APM</td>
</tr>
<tr>
<td>AQP3</td>
<td>Rat</td>
<td>292</td>
<td>Collecting duct</td>
<td>Multiple organs</td>
<td>BLM</td>
</tr>
<tr>
<td>AQP4</td>
<td>Rat</td>
<td>301</td>
<td>Medullary collecting duct</td>
<td>Brain and multiple organs</td>
<td>BLM</td>
</tr>
<tr>
<td>AQP6</td>
<td>Rat</td>
<td>276</td>
<td>Collecting duct</td>
<td>?</td>
<td>VES</td>
</tr>
<tr>
<td>AQP7</td>
<td>Rat</td>
<td>269</td>
<td>Cortex, medulla</td>
<td>Testis, ?</td>
<td>APM ?</td>
</tr>
<tr>
<td>AQP5</td>
<td>Rat</td>
<td>265</td>
<td>Cortical, medulla</td>
<td>Submandibular gland</td>
<td>?</td>
</tr>
<tr>
<td>AQP6</td>
<td>Rat</td>
<td>263</td>
<td>Cortical, medulla</td>
<td>Testis, pancreas, liver, colon, heart, placenta</td>
<td>?</td>
</tr>
<tr>
<td>AQP9</td>
<td>Human</td>
<td>295</td>
<td>Cortical, medulla</td>
<td>Liver, leucocytes, lung, spleen</td>
<td>?</td>
</tr>
</tbody>
</table>

AQP, aquaporin; APM, apical plasma membrane; BLM, basolateral plasma membrane; VES, intracellular vesicles; ?, unknown; +++ significantly regulated; +, regulated; −, not regulated. Note that most of the renal AQPs have been cloned from several species (human, rat, and mouse).
evidence that vasopressin-regulated trafficking of AQP2 represents the cellular mechanism underlying the acute regulation of body water balance by vasopressin, thereby providing support for the original “shuttle hypothesis” proposed by Wade and colleagues (reviewed in Ref. 9) based on studies in toad urinary bladders. Consistent with this, vasopressin treatment of rats in vivo was found to be associated with redistribution of AQP2 to the apical plasma membrane of collecting duct cells, whereas treatment with vasopressin V2 receptor antagonist produced an internalization of AQP2 from the apical plasma membrane to intracellular vesicles and multivesicular bodies (reviewed in Refs. 9 and 12).

Several laboratories have been successful in reconstituting elements of vasopressin regulation of AQP2 in cultured cell systems (2). Through transfection of AQP2 or AQP2-c-myc into MDCK or LLCPK1 cells, increased AQP2 plasma membrane labeling and transcellular water flow have been seen in response to vasopressin or forskolin treatment, whereas AQP2 plasma membrane levels and water transport were reduced in response to removal of vasopressin or forskolin. The studies also indicated that AQP2 may be subjected to recycling during repeated challenges to forskolin or vasopressin.

AQP2 contains a consensus site for PKA phosphorylation in the cytoplasmic carboxy terminus (Ser256). Using AQP2-transfected LLCPK1 cells, it was shown that PKA-mediated phosphorylation of Ser256 is critical for vasopressin-induced trafficking of AQP2 from intracellular vesicles to the plasma membrane (2). Consistent with this, it has been demonstrated that AQP2 is phosphorylated in the PKA consensus site in response to vasopressin treatment of kidney tissue slices (13). Antibodies have recently been developed that selectively recognize AQP2 phosphorylated at Ser256. These antibodies labeled both the apical plasma membrane and vesicles, and it was demonstrated that phosphorylation of this serine was regulated via V2 receptors (4). Thus it appears likely that PKA phosphorylation/dephosphorylation of AQP2 may be involved in the regulated trafficking of AQP2 to and from the plasma membrane (Fig. 2). It remains unknown whether phosphorylation of other serines or threonines (by other
kinases) may be involved in the regulated exocytic or endocytic events as well.

Vesicle-targeting receptors, the so-called SNARE proteins, are believed to play a key role in synaptic vesicle targeting, docking, and fusion. VAMP2, which is a vesicle SNARE, has been found associated with AQP2-bearing vesicles, and, recently, target SNAREs such as syntaxin-4 and SNAP23 have been identified in collecting duct principal cells, using RT-PCR and immunocytochemistry, where they are localized in the apical plasma membrane of collecting duct principal cells. This supports the view that SNARE vesicle-targeting receptors may play a role in vasopressin regulation of AQP2 trafficking (Fig. 2). However, functional data to support this view is awaited.

Studies from the 1970s have made it clear that the cytoskeleton is involved in the regulation of osmotic water permeability by antidiuretic hormone. Recently, dynein, a microtubule-based motor protein, was shown to be associated with AQP2-bearing vesicles (reviewed in Ref. 12). Dynactin was also found to be associated with AQP2-bearing vesicles (reviewed in Ref. 12). Since dynactin is believed to link vesicles via dynein to microtubules, this further supports the view that microtubule-based motor proteins, and associated proteins, may be involved in vasopressin-regulated trafficking of AQP2 (Fig. 2). Actin, together with myosin-1, has also been hypothesized to be involved in AQP2 trafficking.

Regulation of AQP2 protein abundance

The ability of the kidney to increase or decrease the urinary concentrating capacity in response to changes in hydration status is dependent on regulation of solute and water transport in different tubule segments. It has been demonstrated that in the collecting duct there is an adaptational regulation of the osmotic water permeability (9), and several studies have established that this response is associated with changes in the total number of AQP2 water channels per cell (9, 12). Water restriction or chronic vasopressin treatment induces an increase in AQP2 levels that is paralleled by an increase in collecting duct water permeability (9). Conversely, water loading or treatment with V2 receptor antagonists decreases the overall abundance of AQP2. The adaptational changes in AQP2 abundance in turn change the levels of AQP2 available for short-term regulation of trafficking to/from the apical plasma membrane to regulate body water balance. Both vasopressin-dependent and vasopressin-independent regulation are involved in controlling AQP2 expression (Fig. 2). This long-term increase in AQP2 abundance is, at least in part, ascribed to regulation of AQP2 gene transcription, possibly involving a cAMP response element in the 5'-flanking region of the AQP2 gene. Mice that have inherently high levels of cAMP-phosphodiesterase activity and hence low cytosolic levels of cAMP in the collecting...
Table 2. Water balance disorders associated with aquaporin dysregulation

<table>
<thead>
<tr>
<th>Acquired nephrogenic diabetes insipidus</th>
<th>Lithium treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypokalemia</td>
<td>Hypercalcemia</td>
</tr>
<tr>
<td>Postobstructive nephropathy</td>
<td></td>
</tr>
<tr>
<td>Bilateral ureteral obstruction</td>
<td>Unilateral ureteral obstruction</td>
</tr>
</tbody>
</table>

Genetic defects
- Central diabetes insipidus
- Hereditary nephrogenic diabetes insipidus
  - X-linked: Mutations in vasopressin V$_2$ receptor gene
  - Non-X-linked: Mutations in AQP2 gene
    - Diabetes insipidus +/+ severe mice (increased cAMP-phosphodiesterase levels)

Diseases/conditions with water retention
- Congestive heart failure
- Hepatic cirrhosis
- Nephrotic syndrome
- Pregnancy

Other diseases/conditions
- Syndrome of inappropriate antidiuretic hormone
- Secretion/vasopressin escape
- Primary polydipsia
- Chronic renal failure
- Acute renal failure
- Low protein diet
- Age-induced reduction in urinary concentrating capacity

Roles of AQP2 in diseases or conditions with altered water balance

The first demonstration that AQP2 was essential for urinary concentration came from a study by Deen et al. (5). They found mutated and nonfunctional AQP2 in patients with very severe nephrogenic diabetes insipidus (non-X-linked NDI). Subsequently, it was demonstrated that Brattleboro rats, which are vasopressin deficient and have extreme polyuria and therefore have central diabetes insipidus, have reduced expression of AQP2 and very low levels in the apical plasma membrane.

In contrast to the rare inherited forms of diabetes insipidus (central and nephrogenic), acquired forms of nephrogenic diabetes insipidus are much more common. A series of studies has been aimed at testing whether reduced expression and apical targeting of AQP2 might play a role in these polyuric conditions. For this purpose, several classic experimental protocols were used. Prolonged lithium administration to rats causes a 95% downregulation of AQP2 expression and a similar reduction in the apical plasma membrane levels of AQP2. This was associated with the development of extreme polyuria (10), strongly supporting the view that dysregulation of AQP2 plays a fundamental role in the development of polyuria in acquired NDI (10). It was subsequently demonstrated that hypokalemia and hypercalcemia, which are relatively common electrolyte disorders and well-known causes of acquired nephrogenic diabetes insipidus, were also associated with downregulation of AQP2 expression and targeting. In these two conditions, the downregulation of AQP2 was much more modest, as was the polyuria, further supporting a role of AQP2 downregulation. Again, it should be emphasized that it is very likely that these conditions are also associated with other defects, e.g., in solute transport in other segments, that may also contribute to the polyuria.

A relatively common condition associated with impaired urinary concentrating is obstruction of the urinary tract. Experimental bilateral obstruction of the ureters for 1 day was found to be associated with significant downregulation of AQP2 in rats. This downregulation persisted after release of obstruction and was associated with development of prolonged polyuria. Likewise, after 1 day of unilateral ureteral obstruction, a condition in which there are no overall changes in urine production and solute excretion rates, both AQP2 mRNA and AQP2 protein levels were downregulated in the obstructed kidney. In addition, AQP2 levels were moderately reduced in the unobstructed kidney, suggesting that AQP2 downregulation may also be important for the compensatory increase in urine output from this kidney (excreting about twice as much urine to compensate for the obstructed kidney). Solute free water clearance changed in parallel in both the obstructed and unobstructed kidneys in a pattern that matched the reduction in AQP2 expression closely. This further supports the view that AQP2 downregulation, together with other defects, e.g., in solute transport, plays a role in the development of polyuria in postobstructive nephropathy.

In two recent studies it was demonstrated that rats with congestive heart failure and renal water retention have increased levels of AQP2 expression and a marked redistribution of AQP2 water channels with increased targeting to the apical plasma membrane. It is important that this was only seen in rats with severe congestive heart failure and not in rats with compensated heart failure (i.e., in rats with increased left-ventricular end-diastolic filling pressure but no hyponatremia). This supports the view that increased AQP2 expression and targeting, in conjunction with the altered renal handling of sodium, may participate in the retention of water and development of hyponatremia in severe heart failure. Both the increased expression and targeting of AQP2 may be ascribed to increased baroreceptor-mediated vasopressin release. It was recently demonstrated that AQP2 expression levels were also increased in pregnant rats, a condition that is associated with water retention. This raises the possibility that increased AQP2 expression may play a role in the development of water retention in pregnancy as well.
Hepatic cirrhosis is another serious chronic condition associated with water retention. Hepatic cirrhosis can be experimentally induced by chronic administration of carbon tetrachloride. In studies using intraperitoneal administration, cirrhosis was found to be associated with a moderately increased AQP2 protein and mRNA expression. Cirrhosis can also be experimentally induced by ligation of the common bile duct, which can produce a compensated state of cirrhosis with respect to salt and water handling. These rats display an impaired vasopressin-regulated water reabsorption as determined by the reduced effect of V2 receptor antagonists in inducing water excretion. Consistent with this, AQP2 levels were significantly decreased in the rats with compensated cirrhosis. Thus dysregulation of AQP2 may be involved in the dynamic changes in water handling in hepatic cirrhosis.

"Vasopressin escape" describes the condition (physiological or pathophysiological) in which the normal hydroosmotic effect of vasopressin is relieved or reduced. Several experimental studies have been undertaken to elucidate the role of aquaporins in this setting. Rats were chronically infused with 1-desamino-[8-D-arginine]vasopressin (dDAVP) in osmotic mini-pumps and were divided into two groups. One group of rats was water loaded, and the other group was allowed free access to water. Despite the continued administration of dDAVP, water-loaded rats exhibited a marked downregulation of AQP2 and developed polyuria compared with the antidiuretic control rats. Thus the rats escape from the action of vasopressin. This downregulation of AQP2 is likely to represent a physiologically appropriate way to reduce the capacity to reabsorb water and thereby prevent hyponatremia and water intoxication. The signaling transduction pathways involved in this are not well understood, but vasopressin-independent regulation is clearly involved. Thus the existence and potential importance of a vasopressin-independent regulation of AQP2 expression has gained considerable support and is likely to play a physiological and pathophysiological role.

Disturbed renal water handling is a main characteristic of complex renal diseases such as nephrotic syndrome, chronic renal failure, and acute renal failure. A series of different classic experimental models have been used to examine the potential role of aquaporins in these water balance disorders. With respect to nephrotic syndrome, there are defects in the mechanisms responsible both for urinary dilution and urinary concentration. The reasons for these disturbances are incompletely understood. The reduced urinary diluting ability is likely a result of nonosmotic elevation in plasma vasopressin levels, which may then increase free water reabsorption. In rats with puromycin aminonucleoside (PAN)-induced nephrotic syndrome or with adriamycin-induced nephrotic syndrome, an extensive reduction in AQP2 and AQP3 expression was seen, suggesting that the impaired urinary concentrating capacity in nephrotic syndrome could in part be ascribed to this. It was speculated that this response seems to be physiologically appropriate to reduce a further extracellular fluid volume expansion. Chronic renal failure is also characterized by a defect in urinary concentration, and several studies using isolated perfused tubules have disclosed a defect in the collecting duct water handling. Recently, it was demonstrated that AQP2 and AQP3 are severely downregulated, providing a potential mechanism contributing to this collecting defect. Also, ischemia-induced acute renal failure is known to be associated with polyuria of unknown mechanisms. Although clearly severe defects have been disclosed in proximal tubules and thick ascending limbs, the collecting duct also appears to be involved. Two models are generally used: unilateral or bilateral clamping (of the renal pedicle or selectively of the renal artery) for 30, 45, or 60 min followed by 1–5 days of recovery. Aquaporin expression, including expression of AQP2 and AQP3, is significantly reduced after unilateral and bilateral clamping, and this was associated with significant polyuria. These data provide direct evidence that there is a collecting duct defect and implicate a role of aquaporin downregulation in the development of the polyuria. It should be emphasized that recent studies of experimental nephrotic syndrome, chronic renal failure, and acute renal failure have demonstrated that, in addition to the downregulation of aquaporins, there is also dysregulation of a number of solute transporters. This reinforces the important issue that urinary concentration and dilution depends critically on both active transport processes (which are also involved in establishing the driving force for water reabsorption) and on aquaporins for osmotic equilibration. Continued studies of aquaporins, as well as of solute transporters, are likely to provide details to further elucidate the molecular basis for regulation and dysregulation of body water balance.

References
Atrial Natriuretic Peptide: Regulator of Chronic Arterial Blood Pressure

L. G. Melo and U. Ackermann are in the Department of Physiology of the University of Toronto, 1 King’s College Circle, Toronto, ON M5S 1A8, Canada. S. C. Pang is in the Department of Anatomy and Cell Biology, Faculty of Health Sciences, Queen’s University, Kingston, ON K7L 3N6, Canada.

Recent findings in atrial natriuretic peptide (ANP) transgenic and gene knockout mouse models may underlie the etiology of sodium-retaining disorders. With elevated salt intake, ANP-mediated antagonism of the renin-angiotensin system may be overcome, in part, with the introduction of genetic pharmacological receptor antagonists. These difficulties have been overcome, in part, with the introduction of genetic pharmacological receptor antagonists. Recent work in these murine models provides evidence for a role of ANP in chronic regulation of blood pressure and fluid-electrolyte balance. ANP is predominantly synthesized, stored, and secreted in the heart in a regulated fashion by modified myocytes of the cardiac atria. However, in pathophysiological conditions of hemodynamic overload, such as in congestive heart failure, ventricular dysfunction and neurohormone release. The biologically active 28-amino acid peptide is cleaved from the carboxy end of a prohormone and released in response to stretch of the secretory myocytes, consequent to an increase in central venous pressure. On release, ANP exerts its biological effects by interacting with a membrane-bound guanylate cyclase-linked receptor (NPR-A) and subsequently stimulating intracellular cGMP synthesis. A second receptor subtype (NPR-C) is primarily involved in clearance of the peptide from the circulating pool of the peptide. ANP is also synthesized in lesser amounts in some peripheral tissues, in the vasculature, and in the brain. Despite the extensive characterization of acute cardiovascular and renal actions of ANP, progress in elucidating a role for this hormone in chronic regulation of blood pressure and fluid-electrolyte balance was hampered by the lack of suitable experimental models of ANP-induced disease or selective pharmacological receptor antagonists. These difficulties have been overcome, in part, with the introduction of genetic pharmacological receptor antagonists. Recent work in these murine models provides evidence for a role of ANP in chronic regulation of blood pressure and fluid-electrolyte balance.

The earliest evidence that ANP may participate in chronic arterial blood pressure and fluid-electrolyte balance was hampered by the lack of suitable experimental models of ANP-induced disease or selective pharmacological receptor antagonists. These difficulties have been overcome, in part, with the introduction of genetic pharmacological receptor antagonists. Recent work in these murine models provides evidence for a role of ANP in chronic regulation of blood pressure and fluid-electrolyte balance. ANP is predominantly synthesized, stored, and secreted in the heart in a regulated fashion by modified myocytes of the cardiac atria. However, in pathophysiological conditions of hemodynamic overload, such as in congestive heart failure, ventricular dysfunction and neurohormone release. The biologically active 28-amino acid peptide is cleaved from the carboxy end of a prohormone and released in response to stretch of the secretory myocytes, consequent to an increase in central venous pressure. On release, ANP exerts its biological effects by interacting with a membrane-bound guanylate cyclase-linked receptor (NPR-A) and subsequently stimulating intracellular cGMP synthesis. A second receptor subtype (NPR-C) is primarily involved in clearance of the peptide from the circulating pool of the peptide. ANP is also synthesized in lesser amounts in some peripheral tissues, in the vasculature, and in the brain. Despite the extensive characterization of acute cardiovascular and renal actions of ANP, progress in elucidating a role for this hormone in chronic regulation of blood pressure and fluid-electrolyte balance was hampered by the lack of suitable experimental models of ANP-induced disease or selective pharmacological receptor antagonists. These difficulties have been overcome, in part, with the introduction of genetic pharmacological receptor antagonists. Recent work in these murine models provides evidence for a role of ANP in chronic regulation of blood pressure and fluid-electrolyte balance.

References