REVIEW

Vasopressin-independent mechanisms in controlling water homeostasis

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Abstract

The maintenance of body water homeostasis depends on the balance between water intake and water excretion. In the kidney, vasopressin (Vp) is a critical regulator of water homeostasis by controlling the insertion of aquaporin 2 (AQP2) onto the apical membrane of the collecting duct principal cells in the short term and regulating the gene expression of AQP2 in the long term. A growing body of evidence from both *in vitro* and *in vivo* studies demonstrated that both secretin and oxytocin are involved as Vp-independent mechanisms regulating the renal water reabsorption process, including the translocation and expression of AQP2. This review focuses on how these two hormones are potentially involved as Vp-independent mechanisms in controlling water homeostasis.

Journal of Molecular Endocrinology (2009) **43**, 81–92

Introduction

Osmoregulation involves sophisticatedly integrated physiological and behavioral responses to maintain the osmolality of the fluid bathing body cells. The regulation of renal handling of water and electrolytes by the antidiuretic hormone, vasopressin (Vp), and the regulation of water and salt intake by the dipsogenic peptide, angiotensin, are involved in the physiological and behavioral approaches respectively. In recent years, many studies have shown that other hormones also exhibit anti-diuretic functions and these findings add to a growing body of evidence indicating the involvement of Vp-independent mechanisms in regulating renal water reabsorption (Bauman 1965, Jeon *et al.* 2003, Li *et al.* 2006). Secretin (Waldum *et al.* 1980, Chu *et al.* 2007) and oxytocin (Brooks & Pickford 1958, Jeon *et al.* 2003), were previously argued to exert either diuretic or anti-diuretic effects, are therefore candidates in regulating renal functions. This review will focus on how these two hormones are potentially involved as Vp-independent mechanisms controlling water homeostasis.

Current concepts in water regulation

The maintenance of body water homeostasis depends on the balance between water intake and water excretion, with water intake being governed by the

sensation of thirst and the availability of water, and water excretion controlled by the antidiuretic hormone Vp and the medullary osmotic gradient.

Vasopressin

Vp is a pleiotropic peptide which affects a wide range of peripheral and central regulated functions in order to conserve water in the kidney. It controls extracellular fluid osmolality by adjusting the amount of free water excreted by the kidney and the main effect of Vp is found in the collecting duct where it causes insertion of aquaporin 2 (AQP2) water channels onto the apical membrane (Nielsen *et al.* 1993, 1995, Fushimi *et al.* 1997). AQP2 acts as a hydrophilic pore to move water transepithelially from the lumen to interstitium via AQP2 at the apical membrane and AQP3/AQP4 at the basolateral membrane.

In the kidney, Vp binds to a G protein-coupled receptor, Vp type 2 receptor (V2R), on the basolateral membrane of the collecting duct principal cells to stimulate the activity of adenylate cyclase, which subsequently increases intracellular cAMP levels and leads to the activation of protein kinase A (PKA). Activated PKA is then targeted to AQP2-bearing intracellular vesicles (IVs) by PKA anchoring protein 18- δ (Klussmann & Rosenthal 2001), in which it phosphorylates Ser²⁵⁶ of AQP2 (van Balkom *et al.* 2002).

Moreover, activated PKA could also phosphorylate Rho at Ser¹⁸⁸ leading to an attenuation of Rho activity that favors depolymerization of F-actin (Tamma *et al.* 2003). This V2R-mediated signaling leads to the exocytic insertion of AQP-2-bearing vesicles onto the apical plasma membrane (PM), resulting in a high collecting duct water permeability and hence the osmotically driven water movement from lumen to the interstitium, achieving reabsorption of water in the kidney.

To compensate for the hypovolemic or hypernatremic state of the body, Vp also stimulates the expression of AQP2 and affects different regions of the nephron in addition to the stimulation of AQP2 translocation. Studies reported that Vp up-regulates the transcription of AQP2 gene through a cAMP response element in the AQP2 promoter (Hozawa *et al.* 1996, Matsumura *et al.* 1997). In addition, it was shown by Sands (2003), that Vp could assist in the high rates of trans-epithelial urea transport. Such transport is mediated by the PKA phosphorylation of the urea transporter A1 and/or A3, which are located in the terminal collecting duct in the inner medulla. This can bring large amount of urea into the inner medulla to maintain a high interstitial osmolality resulting in maximized urine concentration.

Osmolar gradient between the medullary interstitium and the luminal fluid is the final determination of the amount of water being reabsorbed. The existence of a medullary hypertonic interstitium is ensured by the reabsorption of NaCl against its electrochemical gradient in the thick ascending limb of the loop of Henle. Vp could also induce Na⁺ reabsorption in the thick ascending limb as well as in the cortical and outer medullary collecting ducts, ensuring the existence of a medullary hypertonic interstitium for maximum water reabsorption. Vp exerts the natriuretic response by inducing translocation of Na⁺-K⁺-ATPase from a brefeldin A-sensitive intracellular pool to the basolateral PM in the thick ascending limb and the cortical collecting ducts (Capurro 2001, Feraille *et al.* 2003), thus increasing the abundance of α , β , and γ subunits of the epithelial sodium channel in the cortical collecting ducts (Ecelbarger *et al.* 2000). In addition, Vp also increases phosphorylation of regulatory threonines in the amino terminus of Na-K-Cl cotransporter (NKCC2) and induces the trafficking of NKCC2 in the thick ascending limb (Gimenez & Forbush 2003; Fig. 1).

Collecting duct AQPs

High water permeability in the collecting duct is required for the absorption of water out of the renal tubule. Transepithelial water transport across the collecting duct epithelium is generally believed to

occur by a transcellular route with serial passages across the apical and basolateral PMs. A series of studies over the past ten years have clearly demonstrated that osmotic water transport across the tubule epithelium is chiefly dependent on AQP water channels, which are present in the apical (AQP2; Nielsen *et al.* 1993) and basolateral PM (AQP3, AQP4; Ishibashi *et al.* 1994, Ecelbarger *et al.* 1995, Terris *et al.* 1995). In both human and mouse models, the presence of AQP2 in apical PM was shown to be essential in the urinary concentrating mechanism (Deen *et al.* 1994b), however, only AQP3 null mice were markedly polyuric (Ma *et al.* 2000) and AQP3 null individuals did not suffer any obvious clinical syndromes (Roudier *et al.* 2002).

AQP2

AQP2 is widely expressed in all renal tubule segments. It is most abundant in principal cells of the collecting duct and less so in tubule cells of the connecting tubule or inner medullary collecting duct (IMCD; IMCD cells; Nielsen *et al.* 1993, Loffing *et al.* 2000). Within these cells, AQP2 is primarily found embedded in the apical PM and subapical vesicles, while it is also present in the basolateral PM, particularly in IMCD cells (Marples *et al.* 1995, Nielsen *et al.* 1995, Breton & Brown 1998, Coleman *et al.* 2000).

It is generally agreed that Vp regulates the water permeability of the mammalian collecting duct and hence urine concentration via long term regulation of AQP2 abundance and short term induced translocation of the protein (Brown 2003). To further clarify the role of AQP2 in concentrating urine, a number of mouse models have recently been developed. A mouse knock-in model of AQP2-dependent nephrogenic diabetes insipidus (NDI) was generated; the mouse line was created by using a Cre-loxP strategy to insert a T126M mutation into the AQP2 gene, resulting in blocked delivery of mature AQP2 protein to the apical PM (Yang *et al.* 2001). These knock-in mice generally died within 1 week of birth although they appeared outwardly normal. Other transgenic mouse models have also been developed to examine the role of AQP2 in the adult mouse. One model, developed by Rojek *et al.* (2006), makes use of the Cre-loxP system of gene disruption to create a collecting duct-specific deletion of AQP2, leaving relatively normal levels of expression in the connecting tubule. Another model developed by Yang *et al.* (2006) has an inducible AQP2 gene deletion in the kidney. These cell-specific mutants shared common phenotypes with severe polyuria and decreased urinary osmolality. However, under free access to water, plasma concentrations of electrolytes, urea, and creatinine in knockout mice are comparable with the controls despite polyuria. Apparently, these

Figure 1 An overview of the multiple actions of vasopressin in conserving water in the kidney. In the collecting duct, Vp binds to V2 receptor (V2R) to initiate the synthesis of intracellular cAMP via G_s /adenylyl cyclase (AC). The increased cAMP activates PKA which phosphorylates AQP2. This V2R-mediated signaling pathway eventually leads to exocytotic insertion of AQP2-bearing vesicles onto the apical plasma membrane. In the terminal inner medullary collecting duct (IMCD), Vp acts through PKA to increase the phosphorylation of the UT-A1 urea transporter. Vp exerts a natriuretic effect via the same signaling pathway to induce the translocation of the Na–K–Cl cotransporter in the thick ascending limb (TAL), epithelial sodium channel (ENaC) in the cortical collecting ducts (CCD) and $Na^+K^+ATPase$ in both the CCD and TAL.

transgenic mice have normal renal function but are defective in urinary concentrating ability, thus implicating AQP2 in transcellular reabsorption of water in the collecting duct.

AQP2 regulates urine concentration under the control of Vp, however, there is considerable evidence indicating the presence of Vp-independent mechanisms. For example, at maximal plasma levels of Vp at 10 pM under severe dehydration, osmotic water permeability was at 44% of its maximal value (Star *et al.* 1988), suggesting that factors other than Vp could boost the osmotic water permeability to levels higher than that obtainable by Vp alone. Another group found that hyperosmolality *in vivo* in Vp-deficient Brattleboro rats would also increase expression and trafficking of AQP2 as well as urinary osmolality (Li *et al.* 2006). As indicated in recent studies, secretin and oxytocin are essential components of the Vp-independent mechanism in kidney. Secretin is a classical gastrointestinal hormone and its major function is to stimulate electrolytes and water secretion from the intestine,

liver, and pancreas. Oxytocin is released from the posterior pituitary to stimulate uterine contraction at parturition and mammary gland smooth muscle contraction during lactation. Both hormones have previously been argued to exert either diuretic or anti-diuretic effects and have recently been shown to stimulate AQP2 translocation, hence, are putative Vp-independent mechanisms in controlling water homeostasis.

The pharmacological actions of secretin in the kidney

The role of secretin and its receptor in regulating renal functions has been suggested but not fully substantiated in the past as inconsistent findings regarding the renal functions of secretin have been reported. Earlier studies suggested a diuretic role of this peptide in normal human subjects and dogs (Baron *et al.* 1958). Baron *et al.* (1958) and Alfredo *et al.* (1975) both noted that i.v. injection of secretin caused a rise in urinary

volume and bicarbonate excretion in normal human subjects. Alfredo suggested that secretin had a direct effect on the renal tubule to decrease the reabsorption of water, bicarbonate, sodium, and chloride. Consistent with these results, Waldum *et al.* (1980) also observed a significant increase in urinary water, sodium, calcium, and solute excretion after the infusion of pure natural secretin. It was suggested that the impairment of sodium reabsorption in the renal tubule was the cause. In their study, secretin infusion caused a significant increase in renal plasma flow which may impair tubular sodium reabsorption. A rise in renal plasma flow could either be due to renal vasodilation or to cardiac output, or a combination of both, and earlier studies have showed a vasodilatory effect of secretin. They therefore concluded that the diuretic effect of secretin was most likely contributed by the impairment of sodium reabsorption in renal tubule caused by an increase in renal plasma flow as a result of dilation of the renal vascular bed. A consistent diuretic effect of secretin was also demonstrated in dogs (Dragstedt & Owen 1931). Different secretin preparations were extracted from the mucosa of the first six feet of the hog's intestine and all these preparations were found to produce significant diuresis after i.v. injection into both anesthetized and unanesthetized dogs. A latent period of 20–30 min was observed after the injection, followed by a period of diuresis during which the increased flow varied from 50 to 100%. Another study also demonstrated that i.v. infusion of 0.5 µg/kg min of pure synthetic secretin into conscious dogs resulted in diuresis and significant increases in sodium and potassium output (Barbezat *et al.* 1972).

Earlier reports consistently showed a diuretic effect of secretin, however, two later studies reported contradictory results. One of the studies showed that intravenously administered secretin has an anti-diuretic effect in rats (Charlton *et al.* 1986). The opposing results may be due to the usage of different animal models and/or peptide sources as well as the dosages of secretin being used. Charlton *et al.* argued that the diuresis was due to the reabsorption and renal excretion of secretin-induced secretion of pancreatic juice and bile. His argument was grounded on a previous study that reported that a decreased diuretic effect of natural secretin occurred in humans with chronic pancreatitis. He also suggested that the impurities of natural secretin used should be taken into account as the presence of an agent causing vasodilation has been reported with natural secretin.

Of all these studies on the renal effects of secretin, they concentrated on the pharmacological actions of secretin, leaving its physiological significance to remain uncertain. To elucidate further the renal effect of secretin, transgenic mouse models were recently generated to unfold the physiological action of secretin on

renal water regulation (Chu *et al.* 2007). In this mouse model, exon 10 of the secretin receptor (SCTR) gene was replaced with a PGK-1 promoter-neomycin resistance gene cassette, resulting in a nonfunctional receptor. These SCTR-null mice (SCTR^{-/-}) exhibited polyuria and polydipsia phenotypes; they drank 8.0 ± 0.3 ml water and produced 2.3 ± 0.1 ml urine, compared with SCTR^{+/+} mice, which drank 5.5 ± 0.3 ml water and produced 1.7 ± 0.1 ml urine. The urine osmolality of SCTR^{-/-} mice (1897 ± 59 mOsm/kg H₂O) was lower than that of SCTR^{+/+} mice (2374 ± 57 mOsm/kg H₂O). In addition, SCTR^{-/-} mice produced urine with reduced Na⁺ (SCTR^{+/+}, 152 ± 4.7 mmol/l; SCTR^{-/-}, 124 ± 5.2 mmol/l), K⁺ (SCTR^{+/+}, 349.8 ± 10 mmol/l; SCTR^{-/-}, 285.2 ± 9.4 mmol/l), urea (SCTR^{+/+}, 1339 ± 43.6 mmol/l; SCTR^{-/-}, 1056 ± 34 mmol/l), and creatinine level (SCTR^{+/+}, 5566 ± 312 µmol/l; SCTR^{-/-}, 4504 ± 232 µmol/l) compared with those of SCTR^{+/+} mice. This study strongly supports an antidiuretic function of secretin in rodents.

Distribution of SCTR in kidney

Previous studies regarding the presence of SCTR within the kidney were limited and inconsistent. Ulrich *et al.* (1998) indicated that there was no expression of SCTR in rat kidney using a RNase protection assay, whereas Ohta *et al.* (1992) and Chu *et al.* (2007) reported SCTR protein and transcript expression in rat and human kidneys by immunohistochemical staining and northern blot analysis respectively. Consistent with this, Charlton *et al.* (1986) found high density of [¹²⁵I]-secretin binding sites in the renal medulla by autoradiographic studies. By immunohistochemical stainings using SCTR^{-/-} as negative controls, SCTR was found in the cuboidal epithelium of the collecting ducts and in the simple columnar epithelium of the ducts of Bellini. Additionally, it was also detected in the proximal tubules and the ascending thick segment of the Loop of Henle (Chu *et al.* 2007).

SCT and SCTR are potentially involved as a Vp-independent mechanism conserving water in the kidney

A previous study showed that i.v. administration of secretin caused a dose-dependent decrease in urine output, suggesting that secretin has an antidiuretic action in the kidney (Charlton *et al.* 1986). As microdissection studies demonstrated the presence of secretin-sensitive adenylyl cyclase in kidney membranes, and that secretin is as potent as Vp in homozygous Vp-deficient Brattleboro rats, it was postulated that

secretin could act on its receptors in the renal medulla to decrease urine output through the activation of adenylate cyclase. In this notion, *SCTR*^{-/-} mice were found to have defects in the urinary concentrating mechanism, suggesting that *SCTR* could function to adjust the amount of free water excreted by the kidney (Chu *et al.* 2007). The osmoregulatory role of secretin was further suggested by the observed increase in serum secretin levels during water deprivation (Oektedalen *et al.* 1982). Consistent with this, the plasma secretin level increases 1.60 ± 0.03 -fold in mice under water deprivation, again indicating a role of secretin in water regulation (Chu *et al.* 2007). Plasma secretin concentration was found to increase three to sixfold during prolonged physical stress, resulting in an altered renal function. The increased plasma secretin concentration, however, could be reversed by the administration of hypertonic glucose solution which causes the retention of water (Oektedalen *et al.* 1982).

The transgenic mice study not only sheds light on the urine concentrating ability and the antidiuretic role of secretin, but also provides evidence that secretin could be one of the Vp-independent mechanisms controlling water homeostasis as this antidiuretic effect observed in transgenic mice was shown to be independent of Vp. The study showed that the serum Vp levels of the *SCTR*^{-/-} is comparable with those of the *SCTR*^{+/+} mice under water *ad libitum*. In addition, there are no significant differences in the transcript levels of V2R in *SCTR*^{-/-} and *SCTR*^{+/+} kidney (Chu *et al.* 2007), clearly showing the impaired urine-concentrating ability of *SCTR*^{-/-} mice is not due to impaired response of kidney to Vp stimulation. The same study also indicated that there were significant reductions in the transcript levels of AQP2 and AQP4 in *SCTR*^{-/-} kidneys while less AQP2 expression was triggered in *SCTR*^{-/-} mice under water deprivation. Taken together, secretin is a likely candidate as one of the Vp-independent mechanisms via regulation of AQP2.

Secretin induces translocation and expression of AQP2 under water deprivation

An increasing body of evidence has revealed that secretin may possess a renal effect. However, the key question concerning the mechanisms involved remains to be answered. The transgenic approach, thus, provides additional insight into the physiological action of secretin in water homeostasis and the underlying mechanism by which secretin exerts its activity.

Quantitative real-time PCR revealed significant reductions in the transcript levels of AQP2 and AQP4 (Chu *et al.* 2007) in *SCTR*^{-/-} mice, suggesting that the impaired urine-concentrating ability of *SCTR*^{-/-} mice is at least partly due to the reduced levels of these AQPs.

AQP2, located on the apical membrane, concentrates urine by reabsorbing water, while AQP4, present on the basolateral membrane of the collecting tubules, represents a potential exit pathway for water entering via AQP2. The reductions in both transcript and protein levels of these water channels were consistent with the observed phenotypes developed in *SCTR*^{-/-} animals.

By examining the *in vitro* effects of secretin on the distribution of AQP2 in the inner medullary tubular cells of *SCTR*^{+/+} and *SCTR*^{-/-} mice, secretin was found to induce a dose-dependent increase in both glycosylated and non-glycosylated AQP2 proteins in the PM of medullary tubules in *SCTR*^{+/+} mice (Chu *et al.* 2007). Quantification of the blots revealed a 2.11 ± 0.15 -fold increase in the PM/IV ratio of AQP2 after incubation with 10^{-8} M secretin for 30 min. This effect, however, was not observed in the medullary tubules isolated from *SCTR*^{-/-} mice, clearly indicating the specificity of the actions of secretin via its receptor. We have recently repeated this experiment using inner medullary tubules isolated from the rat kidney in the presence of secretin (10^{-10} – 10^{-8} M). We found that secretin could dose-dependently induce redistribution of AQP2 from the intracellular vesicles to plasma membrane, with a 2.78 ± 0.40 -fold increase in 10 nM secretin (Fig. 2A). The concentration of secretin being used is much higher than the basal plasma secretin concentration in rats (1.8 ± 0.5 pM; Li *et al.* 2001), implicating the effect of secretin on AQP2 relocation at a pharmacological level. Moreover, co-treatment of a secretin antagonist (1 μ M secretin5–27) and a cAMP-dependent PKA inhibitor (5 μ M H89) could both abolish the secretin-induced relocation of AQP2 1.06 ± 0.08 -fold and 1.31 ± 0.12 -fold respectively, indicating that secretin and Vp share similar cAMP/PKA signaling pathway in activating AQP2 trafficking.

The *in vivo* trafficking of AQP2 under chronic hyperosmotic conditions and the *in vitro* effect of secretin on the redistribution of AQP2 in renal tubules have also been investigated. Chu *et al.* (2007) observed significant differences in terms of trafficking and expression of AQP2 between *SCTR*^{-/-} and *SCTR*^{+/+} under water-deprived conditions. Water deprivation for 60 h could trigger a 2.27 ± 0.27 -fold increase in the shift of AQP2 from IVs to the PM and a 5.90 ± 1.06 -fold increase in the AQP2 transcript level in *SCTR*^{+/+} mice. These data suggest that the induced expression and redistribution of AQP2 during water deprivation were at least partially dependent on *SCTR*.

Similar to its role in promoting transepithelial solvent flux in cholangiocytes by activating AQP1 trafficking to the apical membrane, it appears that secretin and Vp alike induce expression of AQP2 under hyperosmotic conditions and stimulate trafficking of this water channel from intracellular vesicles to the plasma membrane in renal collecting tubules.

Figure 2 (A) Secretin induces subcellular redistribution of AQP2 from IV to PM in the inner medullar tubules suspension. Membrane fractions from the rat inner medullar were prepared 30 min post-drug treatment. Western blot and densitometric analysis were performed. Values were calculated from the mean pixel intensity measured from the 35 and 29 kDa bands and were expressed as the fold change in ratios between the intensity of bands from plasma membrane (PM) and intracellular vesicles (IV). ** $P < 0.01$ from the control group. ▲▲ $P < 0.01$ from the 10^{-8} M secretin treatment group. (B) Diagrammatic illustration of the effect of secretin in increasing water reabsorption in the principal cells of the collecting duct. Secretin increases intracellular cAMP levels via binding to SCTR on the basolateral membrane, which is coupled to adenylyl cyclase VI through the heterotrimeric G-protein, Gs. The increased cAMP leads to activation of PKA which subsequently phosphorylates AQP2. This SCTR-mediated signaling leads to the exocytic insertion of AQP2-bearing vesicles into the apical plasma membrane. Secretin also participates in the long-term regulation of AQP2 via the Gs/AC/PKA system, to phosphorylate unknown transcription factors resulting in an increase in both protein and transcript levels of AQP2.

In conclusion, secretin plays a role in regulating body water homeostasis by exerting direct actions in renal system via regulating AQP2 trafficking. Recently, secretin has also been shown to translocate cystic fibrosis transmembrane regulator (CFTR) to the apical membrane in mouse cholangiocytes (Tietz *et al.* 2003). It is possible that secretin modulates renal water permeability via changing concentration of electrolytes in the interstitium by inducing CFTR translocation in a similar way as in cholangiocytes to facilitate active secretion of chloride ions.

Oxytocin

Oxytocin is well-established for its function in female reproduction. Oxytocin and Vp are closely related peptides, both are 9-amino-acid peptide hormones in which seven are identical, and are secreted from the posterior pituitary. Both of them form a cyclic structure via a disulfide linkage between cysteines in the first and sixth positions (Terashima *et al.* 1999). Owing to their similarities in structure and release site, the renal function of oxytocin has also been investigated.

Anti-diuretic action of oxytocin

Oxytocin was reported to exhibit both diuretic and anti-diuretic activities depending on species, dosage, and metabolic status of the animal. In animal studies, oxytocin has consistently been reported to possess an anti-diuretic role at supraphysiological concentrations. Early studies showed that i.v. administration of oxytocin raised the urinary excretion of sodium two to fourfold and enhance glomerular filtration rate in dogs (Brooks & Pickford 1958) and rats (Dicker & Heller 1946). Recent studies using Vp-deficient Brattleboro rats showed that these animals could respond dose-dependently to oxytocin. At an infusion rate of 5 µg/h over a 7-day period, oxytocin completely reversed symptoms of diabetes insipidus (Lyness *et al.* 1985). Similarly, using Brattleboro rats infused with oxytocin by an osmotic minipump for 5 days, marked antidiuresis, increased urine osmolality, and increased solute-free water reabsorption were observed (Li *et al.* 2008). However, the anti-diuretic effect of oxytocin in humans is not proven. An early study showed that i.v. infusion of oxytocin with a dosage ranging from 4 to 200 mU/min produced no measurable anti-diuretic effects and led to insignificant changes of urinary excretion of sodium (Cross *et al.* 1960). On the other hand, another research group found an anti-diuretic effect in 15 out of 17 experiments using the same dosages of oxytocin (Thomson 1960). A recent study reported similar observations in both normal candidates and central

diabetes insipidus (CDI) patients after oxytocin infusion and injection of 1-desamino-8-D-arginine Vp. These subjects showed significant decrease in free water clearance and urine flow and an increase in urine osmolality (Joo *et al.* 2004). In addition, several cases of maternal hyponatremia and water intoxication were reported when oxytocin was employed to induce labor (Ahmad *et al.* 1975), thus, supporting the anti-diuretic action of oxytocin in humans. Further evidence was gathered by infusing graded doses of physiological plasma levels of oxytocin into rats maintained on a sodium-deficient diet, it was found that oxytocin dose-independently increased sodium excretion in rats (Verbalis *et al.* 1991) and osmotic water permeability (Pf) in isolated perfused terminal IMCDs in both Sprague–Dawley and Brattleboro rats. In summary, although there are conflicting findings, previous studies suggest that oxytocin can function physiologically as an antidiuretic hormone (Chou *et al.* 1995b).

Oxytocin produces anti-diuresis by regulating AQP2

A study documented, *in vitro*, that oxytocin caused a marked redistribution of the AQP-collecting duct water channels to a predominantly apical and subapical localization in IMCD cells in Brattleboro rats (Jeon *et al.* 2003). Using immunohistochemical staining, another study demonstrated that AQP2 translocates to the apical plasma membrane after either i.p. or osmotic minipump administration of oxytocin into Brattleboro rats (Li *et al.* 2008). This pattern of AQP2 redistribution was also noted in connecting tubule, cortical collecting duct, and outer medullary collecting duct (Jeon *et al.* 2003). Hence, although Vp is the principal factor in stimulating AQP2 trafficking in the kidney, oxytocin can apparently perform the same function as Vp (Fig. 3). The effects observed by subcutaneous injection of 20 µg oxytocin and 10 µg Vp are comparable, both resulting in an increase in AQP2 expression in the collecting duct in Sprague–Dawley rats (Terashima *et al.* 1999). In Brattleboro rats, oxytocin administration was found to augment both protein and transcript levels of phosphorylated AQP2 and AQP3 (Li *et al.* 2008). Taken together, the anti-diuretic effects observed after pharmacological doses of oxytocin are at least partly due to trafficking of AQP2 and upregulation of AQP2 and AQP3.

Oxytocin exerts its anti-diuretic effect via V2R

Similar to their ligands, receptors for oxytocin and Vp share structural homology and are G protein-coupled receptors. As oxytocin can also bind to Vp receptor but

Figure 3 Signaling cascades involved in Vp/oxytocin-triggered trafficking of AQP2 into the apical cell membrane of collecting duct principal cells. Vp/oxytocin binds to G protein-coupled V2R on the basolateral membrane to stimulate intracellular cAMP production. This initiates the synthesis of intracellular cAMP via the G_s /AC system. The increased cAMP activates PKA which phosphorylates Ser²⁵⁶ of AQP2. Microtubules and microtubule-based motor proteins as well as actin filaments and actin filament binding proteins are necessary for vesicle movement toward the membrane (Tamma *et al.* 2003). This V2R mediated signaling pathway eventually leads to the exocytotic insertion of AQP2-bearing vesicles into the apical plasma membrane, resulting in higher water permeability in the collecting duct and hence water movement from lumen to the interstitium for water reabsorption in the kidney.

with lower affinity (Terashima *et al.* 1999), several studies have suggested the possibility that the anti-diuretic action of oxytocin is mediated via V2R. For example, acute elevation of plasma oxytocin could upregulate AQP2 and downregulated V2R transcripts without affecting the plasma Vp level (Terashima *et al.* 1999). Although both oxytocin receptor and the V2R transcripts were found by RT-PCR in rat collecting duct where the process of urine concentration takes place, *in vitro* microperfusion of rat IMCD showed that only the V2R antagonist [d(CH₂)5(1),D-Ile2,I-le4,Arg8]Vp, but not oxytocin receptor antagonists could block the hydro-osmotic response to 200 pM oxytocin, suggesting actions of oxytocin is mediated by V2R (Chou *et al.* 1995a). This effect of V2R antagonist was repeated in several studies (Pouzet *et al.* 2001, Li *et al.* 2008), together with the data of oxytocin and V2R antagonist on AQP2 translocation in rat collecting duct, provide further evidence that V2R, but not oxytocin receptor, mediates oxytocin's action (Jeon *et al.* 2003).

Disorder of body water homeostasis

Disorders related to water and sodium homeostasis are common problems encountered in clinical practice and can be divided into hyperosmolar and hypoosmolar

disorders. Hyperosmolar and hypoosmolar disorders are characterized by a deficiency and an excess, respectively, of body water relative to body solute. Representatives of such disorders are diabetes insipidus and the syndrome of inappropriate secretion of antidiuretic hormone (SIADH).

Diabetes insipidus

Diabetes insipidus is a syndrome characterized by a failure of urine to concentrate leading to symptoms of polyuria and polydipsia. It is classified into CDI and NDI depending on whether it is a result of decreased secretion of Vp or an impaired response of the kidney to Vp, respectively. CDI is due to the disruption of the hypothalamic–neurohypophysial axis by a variety of acquired and congenital causes, all of which lead to insufficient production of Vp, while NDI is caused by a reduced or absent response to Vp. The genetic forms of NDI are results of mutations in the genes coding for the V2R or AQP2. Among all NDI patients, 90% have various mutations in the V2R gene, which resides on Xq28 and hence is prevalent in males. The remaining 10% patients have mutations of the AQP2 gene, which is situated on 12q13 and thus is inherited in an autosomal manner (Deen *et al.* 1994a, Saito *et al.* 1995). For the X-linked congenital NDI, V2R gene

mutations can be divided into five different classes according to their cellular fate (Fig. 4; Robben *et al.* 2005). Class I leads to unstable RNA, resulting in the formation of an incomplete protein. Receptors in Class II have protein misfolding leading to endoplasmic reticulum (ER) retention and hence degradation within endosomes. Class III mutations produce receptors that are not capable of transducing signals via G proteins. Class IV mutants are unable to bind Vp. Finally, Class V mutations lead to missorting of receptors in the cell. All these mutations lead to the absence of functional receptors on cell surface and hence impairment in water reabsorption.

Autosomal recessive and dominant NDI

For the remaining 10% patients of NDI, the disorder can be either inherited as an autosomal recessive or as an autosomal dominant trait. The disease stems from mutations in AQP2 gene which is mapped to 12q13 (Deen *et al.* 1994a, Saito *et al.* 1995). Of these patients, more than 90% are autosomal recessive in which mutant AQP2 proteins are misfolded, trapped in the ER, and subject to rapid proteasomal degradation (Deen *et al.* 1995, Mulders *et al.* 1997, Tamarappoo & Verkman 1998, Marr *et al.* 2001, 2002, Lin *et al.* 2002). Autosomal dominant inheritance is the least prominent form of NDI. Expression studies revealed that AQP2 in these patients are properly folded, however, due to the mutation, the interaction with the wild type AQP2 cause

the missorting of the wild type-AQP2/mutant complex (Mulders *et al.* 1998, Kamsteeg *et al.* 1999, 2003, Kuwahara *et al.* 2001). Mutations are found within the C-terminal tail of the protein (Robben *et al.* 2006), revealing the importance of this segment in the trafficking of AQP2.

Syndrome of SIADH

SIADH, or syndrome of inappropriate secretion of antidiuretic hormone, frequently occurs in hospitalized patients, and is characterized by hyponatremia and the production of concentrated urine. Studies of Vp release in response to hypertonic saline infusion in patients with SIADH reveal 4 patterns of abnormal Vp secretion (Multz 2007). Type A, the most common, occurs in ~40% of patients and involves excessive, erratic and ectopic secretion of Vp unrelated to plasma osmolality. Type B found in ~30% of patients is characterized by continued water excretion at a lower set point of plasma osmolality. Type C, also occurs in ~30% of patients, is defined by a constant leak of Vp. The abnormality is possibly due to a loss of inhibitory osmoregulatory mechanism or damage to neurohypophysis mechanisms. For type D (5–10%), the cause is not completely understood. In this type of patient, normal osmoregulation of plasma Vp was observed and some of them, in particular infants, appear to suffer nephrogenic syndrome of inappropriate antidiuresis, which is a recently described genetic disease caused by V2R activating mutations resulting in

Figure 4 Schematic representation of five different classes (I–V) of V2R mutation in nephrogenic diabetes insipidus (NDI). Class I mutations lead to unstable RNA, resulting in the formation of incomplete protein. Class II mutations involve endoplasmic reticulum (ER) retention of full-length proteins. Class III mutations result in inefficient G protein-coupling, Class IV mutants are unable to bind Vp. Finally, Class V mutations lead to missorting of proteins.

hyponatremia (Feldman *et al.* 2005). The clinical presentations resemble those typically observed in patients with SIADH but with undetectable arginine vasopressin (AVP) levels. In other type D patients, it may be due to abnormal control of AQP2 in renal collecting tubules (Verbalis *et al.* 1998).

Future perspective

A study showed that *SCTR*^{-/-} mice display pathological symptoms of NDI. Histological examination showed that *SCTR*^{-/-} mice exhibited abnormalities in the renal cortex and the medulla, characterized by increased mesangial area, enlarged urinary space, and frequent tubular dilation and hypertrophy in the collecting tubules of the medullary region. Quantification by real-time PCR revealed significant increases in the transcript levels of interleukin-10, tumor necrosis factor α , E-selectin, and osteopontin which are the proinflammatory cytokines indicating the inflammation and cell recruitment in diabetic nephropathy (Chu *et al.* 2007). Secretin has also been reported to cause an increase in insulin secretion (Enk 1976). In both normal and obese non-diabetics groups, i.v. injection of secretin elicits an increase in insulin concentrations in the cubital vein (Enk *et al.* 1976). In another study, secretin infusion was found to augment immunoreactive insulin in the blood and improve glucose tolerance (Dupre *et al.* 1975). Similar results have also been reported in a transgenic mice study in which a higher blood glucose level was observed in *SCTR*^{-/-} mice (Chu *et al.* 2007). The reported effects of secretin on insulin secretion together with the pathological features observed in the kidneys of *SCTR*^{-/-} mice all point to abnormalities in the production and/or release of this hormone, as well as the disturbance of its receptor, which may manifest into the renal and metabolic perturbations observed in diabetes and SIADH. The anti-diuretic action of secretin and the pathological symptoms of NDI observed in *SCTR*^{-/-} mice suggest that dysfunction of secretin and receptor axis could be a class of NDI. In summary, in a growing body of evidence, reviewed here, secretin can trigger intracellular redistribution of AQP2 to the apical membrane and therefore serve as a potential candidate in treating X-linked NDI with defective V2R signaling. Hence, further investigation is needed to elucidate the potential role of secretin as a target for prevention and/or therapeutic intervention of these diseases.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work was supported by a research grant from Research Grant Council HKU 7501/05 M and 7566/06 M to BKC Chow.

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Received in final form 16 March 2009

Accepted 23 March 2009

Made available online as an Accepted Preprint 23 March 2009