Recent Insights Into the Coordinate Regulation of Body Water and Divalent Mineral Ion Metabolism

MICHELLE A. BAUM, MD, H. WILLIAM HARRIS, MD, PHD

ABSTRACT: Traditionally, arginine vasopressin modulation of renal water, sodium, and urea excretion has been considered somewhat in isolation from factors that control divalent mineral ion homeostasis. Similarly, previous considerations of divalent mineral ion metabolism have focused mainly on the role of hormones, eg, parathyroid hormone and various forms of vitamin D, as principal modifiers of renal calcium handling. Recent data, however, have now suggested the existence of novel linkages that coordinate control of water and divalent mineral ion homeostasis. This article summarizes these data and highlights the fundamental roles of the extracellular calcium polyvalent cation-sensing receptor (CaR) as an integrator of water and divalent mineral ion homeostasis on a cellular, organ-specific, and whole-body basis. Organs where CaRs may integrate water and divalent mineral ion metabolism include endocrine tissues that express CaRs, the brain, various nephron segments of the kidney, bone, and the gastrointestinal tract. These new data suggest that considerable regulatory overlap exists between water and divalent mineral ion homeostasis. KEY INDEXING TERMS: Divalent ion metabolism; Fluids and electrolytes; Kidney; Vasopressin. [Am J Med Sci 1998;316(5):321–328.]

Modulation of renal ion and water excretion by the peptide hormone arginine vasopressin (AVP) is a fundamental mechanism that has been highly conserved throughout evolution, for regulating total body water content.[1,2] The interaction of AVP with kidney epithelial cells has been investigated for over 50 years. Research in the last decade (1988–1998) has focused not only on AVP's regulation of the apical membrane water permeability of the inner medullary collecting duct (IMCD) but also on how various other regulatory factors (including other peptide and steroid hormones and prostaglandins) modulate AVP-elicited changes in IMCD transport.[2,3] Present data demonstrate that IMCD cells are the major intrarenal target for AVP action, and also possess a highly complex cellular machinery to integrate other extracellular signals that together preserve body homeostasis.

The IMCD is located in the deepest portions of the renal medulla; it is surrounded by a hypertonic medullary interstitium generated by multiple elements that together constitute the countercurrent multiplier system of the kidney medulla. In the absence of AVP stimulation, IMCD cells exhibit low permeabilities to sodium, urea, and water, and permit the excretion of large volumes of hypotonic urine formed during intervals of water diuresis. In contrast, during intervals of antidiuresis, AVP stimulation causes rapid increases in water, urea, and sodium permeabilities of the apical membranes of IMCD cells. This process allows for renal reclamation of both water and a portion of the filtered solutes.[4,5]

Traditionally, AVP modulation of renal water, sodium, and urea excretion has been considered somewhat in isolation from factors that control divalent mineral ion homeostasis.[1,2] Similarly, previous investigations of divalent mineral ion metabolism have focused mainly on the roles of hormones such as parathyroid hormone (PTH) and various forms of vitamin D as principal modifiers of renal calcium handling.[6] Recent data have now suggested, however, the existence of novel linkages that coordinate control of water and divalent mineral ion homeostasis. The purpose of this article is to summarize these data and highlight the fundamental roles of the extracellular calcium polyvalent cation-sensing receptor (CaR) as an integrator of water and divalent mineral ion homeostasis on a cellular, organ-specific, and whole-body basis. The new data suggesting that considerable regulatory "overlap" exists between water and divalent mineral ion homeostasis is diagrammed in Figure 1.

From the Division of Nephrology, Children's Hospital, Boston, Massachusetts.

Correspondence: H. William Harris, MD, PhD, Division of Nephrology, Children's Hospital, 300 Longwood Avenue, Boston, MA 02115.

Email: harris@hub.tch.harvard.edu

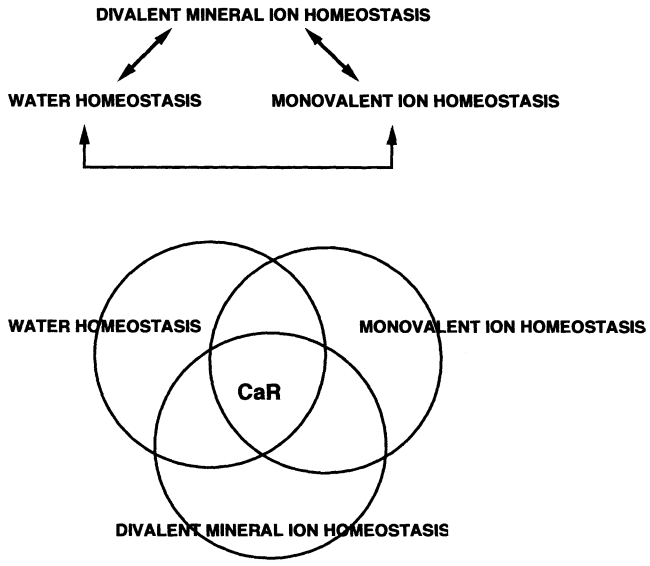

Figure 1. Comparison of diagrammatic schemes of the interrelationships between total body water and divalent mineral ion homeostasis. Upper panel: Traditionally, divalent (Ca^{2+}, Mg^{2+}) mineral ion homeostasis, water homeostasis, and monovalent (NaCl, K) ion homeostasis have been considered as being controlled by separated interconnected processes. Lower panel: Recent data suggest that water, divalent ion, and monovalent ion homeostasis may be more accurately displayed as overlapping circles where the calcium polyvalent cation-sensing receptor (CaR) provides modulatory input into all three areas of body homeostasis.

CaR Acts as a Primary Regulatory Element to Control Divalent Mineral Ion Homeostasis

Divalent mineral ions such as Ca^{2+} and Mg^{2+} have been demonstrated to play critical roles in many cellular functions.[6,7] Research by multiple investigators has detailed the importance of Ca^{2+} in numerous intracellular signalling pathways.[8] In most cells, intracellular Ca^{2+} is maintained by active energy-dependent transport mechanisms at concentrations (nanomolar–micromolar) that are generally at least 1000-fold lower than extracellular Ca^{2+} concentrations (1–2 mmol).[8] Moreover, the Ca^{2+} concentration of the extracellular fluid compartment of most animals, including humans, is also maintained within a very narrow concentration range (1–2 mmol). Maintenance of the Ca^{2+} concentration of the extracellular fluid compartment necessitates the interaction of several different tissues, including the parathyroid, kidney, intestine, and bone.[6] It has now been demonstrated that the CaR permits cells of these various organ systems to "sense" and respond to minute changes in extracellular Ca^{2+} concentrations.[9,10]

Previous efforts to investigate how the parathyroid gland senses alterations in extracellular Ca^{2+} concentrations revealed that the activation of specific intracellular G-protein coupled receptor signaling pathways enabled parathyroid cells to respond to minute changes in serum Ca^{2+} concentrations.[11] Expression studies using *Xenopus* oocytes injected with bovine parathyroid RNA demonstrated that exposure of oocytes to increases in extracellular Ca^{2+} concentrations similar to those present in mammalian serum (1–5 mmol) activated phospholipase C and resulted in increases in inositol triphosphate (IP3), as well as release of Ca^{2+} from intracellular stores within the oocyte.[12] This response was also elicited by other divalent cations such as Mg^{+2} (activation occurring in the 5–50 mmol range) and trivalent (Gd^{3+}) and polyvalent (neomycin) cations that provoked identical responses at nanomolar–micromolar concentration ranges. Using this system, Brown et al[12] cloned a seven membrane-spanning G-protein coupled cell surface receptor from bovine parathyroid mRNA (BoPCaR). Structural analyses revealed that BoPCaR consisted of three major domains, including a large extracellular amino terminal domain with several regions containing clusters of negatively charged amino acids that could serve as binding sites for calcium and other cations, a central domain with seven membrane-spanning regions characteristic of the G-protein family of receptors, and an intracytoplasmic carboxyl terminal domain. Expression of BoPCaR in *Xenopus* oocytes demonstrated that this novel CaR sensed alterations in divalent, trivalent, and polyvalent cations in a pattern identical to that exhibited with parathyroid mRNA.

Subsequent investigations have revealed that CaRs are present in multiple organs that contribute to body calcium homeostasis. These include:

- Calcitonin-producing cells of the thyroid, where CaR may regulate calcitonin secretion[13]
- Myenteric plexi and basolateral membranes of epithelial cells of gastric mucosa, where CaR may modulate ion secretion and GI motility[14]
- Hippocampal neurons and nerve terminals, where CaR appear to modulate a variety of nerve cell functions[15,16]
- Distal convoluted tubule, where CaR may interact with apical Ca^{2+} channels to modulate either Ca^{2+} or Mg^{2+} reabsorption by this nephron segment[17]
- Bone osteoclasts, where CaR may mediate multiple osteoclast functions.[18]

The role of CaRs to regulate renal divalent mineral ion reabsorption has been best established in studies of the kidney thick ascending limb (TAL). Molecular cloning of a rat kidney CaR (RaKCaR), demonstrating that it shares greater than 90% amino acid homology with BoPCaR[19] and is localized to the basolateral membrane of the cortical TAL,[17,20] has enabled detailed studies of how RaKCaR regulates reabsorption of Ca^{2+} and Mg^{2+} by the TAL. A combination of epithelial cell transport studies[10,21,22] as well as molecular cloning of both the Na,K,2Cl co-

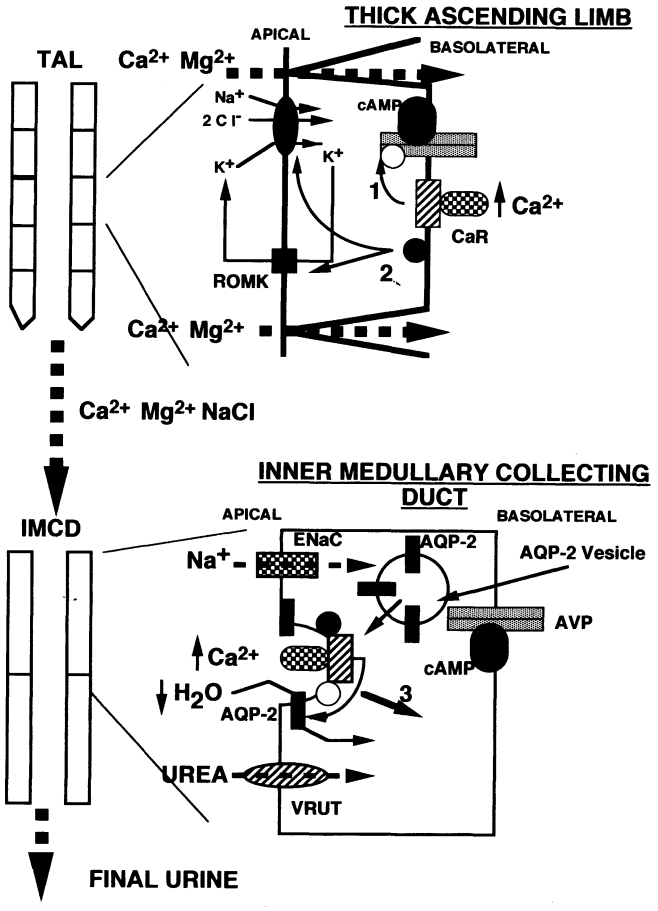

Figure 2. Summary of key roles for CaR in interactions with principal components of NaCl, Ca^{2+}, Mg^{2+} transport of the thick ascending limb of Henle (TAL) and AVP-elicited water and urea transport in the inner medullary collecting duct (IMCD). In TAL, Ca^{2+} and Mg^{2+} reabsorption is driven by a lumen-positive potential created by monovalent ion transport via the Na,K,2Cl cotransporter and ROMK. Elevations in basolateral Ca^{2+} activate TAL CaR that reduces ion transport by TAL, either through pathways involving G_i proteins (#1) that interact with basolateral peptide hormone receptors in TAL, or through pathways involving Gq proteins (#2) that reduce TAL ion transport via arachidonic acid intermediates. Reduction of TAL NaCl, Ca^{2+}, and Mg^{2+} transport increases delivery of these ions to IMCD. Acutely, an increase in luminal Ca^{2+} concentration resulting from AVP-elicited water transport activates a luminal CaR, which reduces AVP IMCD water transport (#3) and prevents excessive increases in luminal Ca^{2+} concentrations that would cause renal stone formation.

transporter[23] and renal outer medullary potassium channel ($ROMK^+$),[24] have established the interdependence of TAL NaCl transport and reabsorption of divalent cations. As shown in Figure 2, active transepithelial transport of NaCl accompanied by recycling of K^+ via the apical ROMK channel generates a lumen-positive potential in the TAL that drives the transepithelial reabsorption of both Ca^{2+} and Mg^{2+}. From its basolateral location, CaR is believed to sense alterations in peritubular (serum) divalent cation concentrations. Activation of this basolateral CaR in TAL reduces both AVP and PTH-stimulated cyclic adenosine monophosphate (cAMP) accumulation, probably through interactions of CaR and basolateral adenylate cyclase via an inhibitory G protein.[25] In addition, other studies suggest that CaR may also activate intracellular signalling pathways in TAL cells involving arachidonic acid intermediates that inhibit K^+ recycling by TAL.[26] Reduction of apical membrane recycling of K^+ via ROMK will further reduce both NaCl and divalent cation reabsorption by TAL. Thus, modulation of TAL NaCl and divalent cation reabsorption by its basolateral CaR provides for integration of monovalent (NaCl) and divalent (Ca^{2+} and Mg^{2+}) mineral ion homeostasis on a cellular level. Moreover, as discussed later, CaR modulation of TAL transport is also integrated with alterations in the urinary concentrating capacity of the kidney via alterations in medullary tonicity. These data suggest an explanation for the changes in renal tubular sodium and calcium transport observed in both patients and rats with hypercalcemia.[27,28] The importance of TAL solute transporters as principal elements in divalent mineral ion homeostasis has been further demonstrated by reports showing that Bartter syndrome is caused by mutations in the Na,K,2Cl gene[29] as well as ROMK.[30,31] Studies of the developmental expression of CaR in the TAL show that CaR expression is increased as postnatal rats are exposed to variations in solute and water intake.[20]

Strong support for a key role of CaRs in the regulation of calcium homeostasis has been provided by analyses of several mutations of the CaR gene localized to human chromosome 3 that cause well-known human clinical disorders of calcium homeostasis.[32] Familial hypocalciuric hypercalcemia (FHH) and neonatal severe hyperparathyroidism (NSHPT) are the heterozygous and homozygous forms, respectively, of disorders that demonstrate an altered set point for Ca^{2+}-regulated PTH release. Patients with FHH are generally asymptomatic and despite their mild to moderate hypercalcemia manifest neither the expected hypercalciuria nor low serum PTH levels.[32] In contrast, NSHPT is a life-threatening neonatal disorder characterized by extreme hypercalcemia and bone demineralization where patients do not survive without parathyroidectomy. Studies of CaR gene structure and expression studies of CaRs possessing these mutations demonstrate that FHH and NSHPT families possess inactivating (loss of function) point mutations of the CaR gene that account for their specific alterations in calcium homeostasis.[32–34]

Another inherited human disorder of calcium homeostasis is autosomal dominant hypocalcemia (ADH).[35] These patients manifest asymptomatic hy-

pocalcemia with a decreased or inappropriately normal PTH. Similar studies of CaR gene structure and CaR protein function in these patients reveal point mutations that produce an activation or gain in function of CaR protein.[33,35]

Detailed studies of alterations in renal tubular handling of divalent cations in patients with FHH, NSHPT, and ADH provide further support for the role of CaRs as modulators of renal tubular ion transport. For example, FHH patients manifest hypocalciuria despite persistent hypercalcemia, even after parathyroidectomy.[36] As compared to normal controls, FHH patients exhibit little increase in renal sodium excretion in response to calcium loading and exhibit a normal ability to concentrate their urine during intervals of dehydration.[37] In a similar manner, ADH patients demonstrate hypercalciuria despite persistent hypocalcemia. Treatment of hypocalcemia in ADH patients with vitamin D supplements produces an increase in renal Ca^{2+} excretion, development of an AVP-resistant urinary concentrating defect, and, in some cases, formation of nephrocalcinosis and kidney stones.[37] Thus, renal tubular transport mechanisms in FHH and ADH patients seem to manifest alterations in renal calcium handling, which suggests that the CaRs present in their kidney tubules also exhibit increased set point (loss of function) or decreased set point (gain of function) identical to those present in parathyroid tissue CaR.[10]

AVP Modulates Apical Membrane Water Permeability of Rat IMCD by Alterations in the Location, Content, and Structure of Aquaporin-2 Water Channels

Molecular cloning of the aquaporin-2 (AQP2), -3, and -4 water channels,[38,39] as well as the AVP-regulated urea transporter (VRUT)[40] has led to a rapid increase in knowledge linking the large body of physiologic and morphologic data published previously to new studies of AVP action. AQP2, AQP3, and AQP4 proteins are expressed in rat IMCD and are hypothesized to participate in AVP-elicited transepithelial water permeability (Pf). As described below, the role of AQP2 is well established. A large body of data obtained from rats[41–43] and from patients with nephrogenic diabetes insipidus possessing mutant AQP2 proteins,[44,45] show that AQP2 is expressed exclusively in AVP-responsive cells of the collecting duct. AQP2 is localized to subapical vesicles undergoing fusion and retrieval from the apical membrane and to the basolateral membranes of IMCD cells, especially after intervals of "conditioning" that up-regulate AQP2 expression.[42,46–48] In contrast, AQP3 is localized exclusively to IMCD basolateral membranes, where it may provide a common exit pathway for water and urea entering the cell via IMCD apical membrane AQP2 water channels and VRUT, respectively.[49,50] AQP4 is also localized to IMCD basolateral membranes and may function in a fashion similar to that hypothesized for AQP3.[51,52] Thus, to permit AVP-responsive transepithelial water and urea transport, IMCD cells must maintain distinct, highly polarized distributions for AQP2, AQP3, AQP4, and VRUT via cellular sorting machinery that efficiently routes each of these membrane proteins to their respective membrane domains. As discussed later, the divergent carboxyl terminal domains of AQPs likely contain important targeting signals that direct each AQP along its appropriate pathway.

Studies performed in rats have identified at least two major mechanisms that regulate apical membrane Pf in IMCD. As shown in Figure 2, AQP2-containing vesicles are clustered in the immediate vicinity of the apical membrane in the absence of AVP stimulation. The binding of AVP to its basolateral V_2 receptor activates adenylate cyclase via a Gs protein and increases intracellular cAMP.[1,2] Increases in cAMP cause insertion of AQP2 water channels into the IMCD apical membrane and permit the passive equilibration of water, but not other ions or solutes, across extremely narrow transmembrane pores present in AQP2. Removal of AVP stimulation as well as other stimuli cause retrieval of AQP2-containing membrane via vesicle endocytosis into a specialized endocytic compartment and the return of apical membrane Pf to low baseline levels.[1]

A combination of biochemical studies using purified AQP2 endosomes from rat IMCD,[53] as well as transfection experiments expressing AQP2 mutants in both *Xenopus* oocytes[54] and cultured cells,[55,56] have provided evidence for the role of cAMP-dependent protein kinase A (PKA) phosphorylation of serine 256 of rat AQP2 in the regulation of the apical membrane trafficking of AQP2. Recent data[57] have suggested that both PKA and protein phosphatase PP2B or calcineurin may be bound to the cytoplasmic surface of AQP2 vesicles by PKA anchoring proteins (AKAPs) similar to that described for PKA-regulated ion channels in neurons. Studies in renal epithelia have demonstrated that both the fusion and the retrieval of AQP2 vesicles is a dynamic process occurring throughout AVP stimulation.[1,58] Therefore, the resulting IMCD membrane Pf represents the sum of vesicle fusion and retrieval at any given moment. These data have established vesicle-mediated AQP2 trafficking as the major regulator of ADH-elicited Pf.

A second AQP2 regulatory mechanism termed "conditioning" occurs at the transcriptional level and alters the steady-state levels of AQP2 mRNA and protein within ADH-responsive cells. Both AQP2 protein and mRNA are increased in IMCD upon exposure of rats to stimuli that activate renal water conservation mechanisms, including: (1) chronic AVP stimulation,[43,46,59] (2) water deprivation,[42,43,48] (3) glucocorticoids,[60] and (4) congestive heart failure.[61,62] In contrast, maneuvers that cause defects in

urinary concentration, including low-protein diets,[63] hypokalemia,[64] lithium exposure,[65] urinary tract obstruction,[66] and chemically induced nephrotic syndrome,[67] all reduce IMCD AQP2 content. Recent studies have provided a partial explanation for these alterations in AQP2 mRNA via activation or repression of both cAMP regulatory and hypertonicity-responsive elements in the promoter region of the AQP2 gene.[68] Thus, the "conditioning" phenomena observed in IMCD is a long-term response to prolonged intervals of either heightened or diminished AVP stimulation that occur during water deprivation or diuresis, respectively. Similar alterations in the cellular content of VRUT have been demonstrated after alterations in glucocorticoid administration[69] and low-protein diets[70] in rats.

CaRs May Integrate Water and Divalent Mineral Ion Homeostasis Within IMCD Cells

Although little significant transepithelial transport of Ca^{2+} or Mg^{2+} occurs in IMCD as compared to TAL,[6] the luminal concentrations of these divalent cations undergo significant increases as a result of AVP-elicited water reabsorption. Under conditions of normal hydration in humans, average IMCD luminal Ca^{2+} concentrations have been estimated as 2 mmol.[71] Elevated IMCD luminal Ca^{2+} concentrations occurring during intervals of either antidiuresis or chronic hypercalcemia likely contribute to the formation of Ca^{2+}-containing renal stones and nephrocalcinosis.

Optimal preservation of the structural and functional features of IMCD require special procedures because IMCD cells are chronically adapted to the hyperosmotic conditions of the renal medulla. Because the use of standard fixation and preservation techniques may have overlooked the presence of an IMCD CaR, in initial attempts to characterize CaR's distribution in rat kidney, our laboratory performed immunocytochemistry analyses of optimally preserved rat IMCD using identical anti-CaR probes to determine if CaR was present in IMCD where it might provide a sensing mechanism to integrate divalent mineral and water metabolism. These studies revealed abundant CaR in both rat IMCD and human collecting duct where CaR was present in a distribution similar to that displayed by AQP2.[72] Immunoblotting of highly purified AQP2-containing endosomes from IMCD[53] revealed specific CaR protein bands as well as the presence of both stimulatory (G_q/G_{a11}) and inhibitory ($G_{\alpha i1,2}$ and $G_{\alpha i3}$) G proteins that have been suggested as key signal transduction elements in CaR signaling pathways.[73] Northern blot analyses of poly A^+ RNA from inner medulla revealed a prominent 7-kb CaR transcript. These data suggest that CaR protein is present in the apical membrane of IMCD cells.

Linkage of CaR action to modulation of AVP-elicited P_f was provided by experiments using isolated perfused rat IMCD tubules.[72] Acute increases in IMCD luminal calcium concentrations from 1 to 5 mmol produced a rapid (<10 min) reversible 30% reduction in AVP-elicited P_f.[72] Identical reductions in AVP-elicited P_f were obtained upon substitution of the CaR agonist neomycin for Ca^{2+} under conditions where AVP-elicited urea transport was not altered significantly (P_{urea}). Taken together, these data suggest the presence of an apical CaR in IMCD that is capable of responding to alterations in luminal calcium by modulation of AVP-elicited P_f. This apical membrane CaR may provide a mechanism for the modulation of AVP-elicited water transport in IMCD, integrating IMCD water transport with luminal Ca^{2+} delivery by the TAL and distal tubule.

As described previously, the TAL provides a major site for Ca^{2+} and Mg^{2+} reabsorption in the mammalian nephron. Simultaneous reductions in TAL reabsorption of NaCl, Ca^{2+}, and Mg^{2+} result in the delivery of increased concentrations of these ions to the IMCD. If AVP increased AQP2-mediated P_f in IMCD under conditions of chronic hypercalcemia, levels of urinary calcium might exceed saturation and greatly increase the risk of renal stone formation. Previous studies by multiple laboratories have demonstrated that hypercalcemia and hypercalciuria produce an AVP-resistant state of nephrogenic diabetes insipidus in both rats and humans.[27,74] Careful investigations by Berl et al[75,76] using dihydrotachysterol (DHT) administration to induce chronic hypercalcemia in rats have revealed that this urinary concentrating defect is characterized by a prostaglandin-independent resistance to exogenous AVP as well as a decreased solute content of the inner medulla. Studies of isolated rat kidney nephron segments demonstrate that exposure to elevated peritubular (basolateral) Ca^{2+} inhibits AVP-elicited cAMP generation by the TAL, but is without effect in medullary collecting duct.[25,76] These data suggest that elevated extracellular Ca^{2+} acts by alteration of AVP-elicited water transport at a point after the generation of cAMP.

To determine how chronic hypercalcemia alters AVP-elicited water and urea transport and AQP2, VRUT, and CaR proteins in IMCD, our laboratories have recently performed a combination of transport measurements and biochemical characterization studies in IMCD isolated from DHT-treated hypercalcemic rats.[77] Unlike matched control tubules from normocalcemic rats, isolated perfused IMCD tubules from hypercalcemic rats displayed no significant increases in P_f after exposure to either 100 pM or 10 nmol AVP. In contrast, both basal and AVP-elicited P_{urea} were significantly increased as compared to controls. Quantitative immunoblotting analyses of IMCD proteins revealed an 87% reduction in IMCD AQP2 protein in hypercalcemic rats as compared to

controls. However, no significant alterations in either IMCD AQP2 mRNA or the apical distribution of AQP2 protein, as determined by immunocytochemistry, were observed. In contrast, IMCD content of VRUT protein was increased by approximately 41% as compared to controls. These data suggest that alterations in IMCD transport and protein content occurring in hypercalcemic rats may constitute an adaptive response promoting renal excretion of Ca^{2+} under conditions that would minimize the possibility of kidney stone formation. These data may provide an explanation for the selective decrease in nonurea solutes that provide for an overall reduction in medullary solute content in hypercalemic DHT-treated rats.[28,75]

These changes in hypercalcemic rats were accompanied by specific alterations in the electrophoretic mobility of the IMCD CaR protein as detected by immunoblotting. Although the nature of these specific changes in CaR's electrophoretic mobility elicited by DHT treatment are unknown at present, they likely relate to alterations in CaR's associations with itself and perhaps other proteins.[78] Future studies will focus on alterations in IMCD function and content in response to hypercalcemia that may be mediated via CaR signal transduction events.

Further localization studies of CaR expression in brain tissue have provided additional evidence suggesting that CaR provides linkages between divalent mineral ion and water homeostasis. Recent data by Rogers et al[79] have revealed high levels of CaR in several regions of the brain that modulate thirst and appetite regulation. The subfornical organ (SFO) of the brain expresses abundant CaR and has been shown to regulate angiotensin II-mediated drinking behavior and to possess connections to hypothalamic nuclei responsible for AVP release. Mechanisms that produce increased drinking behavior of hypercalcemic animals and humans may include the responses to renal water losses resulting from nephrogenic diabetes insipidus, as already discussed, as well as stimulation of thirst via stimulation of these CaR-expressing cells in the SFO. Similar studies have also localized CaR to the area postrema which is an important nausea- and appetite-regulating center of the brain.[34,79] It is possible that stimulation of CaR under conditions of hypercalcemia causes a decrease in ingestion of solute and calcium loads in the form of foods, further facilitating excretion of excess body Ca^{2+}. Future studies will be required to demonstrate the functional significance of CaR localized to these areas of the brain, but present data suggest that brain CaR's may play an important role in integrating the thirst and appetite responses of the body to maintain water and divalent mineral homeostasis.

Summary and Future Directions

Recent studies of the expression patterns and function of CaRs suggest that these divalent cation-sensing proteins provide the molecular basis for a series of coordinated responses involving multiple organ systems that are responsible not only for regulation of the Ca^{2+} concentration of the extracellular fluid compartment in many organisms, but also for integration of divalent mineral ion and water homeostasis. Alterations in extracellular Ca^{2+} concentrations activate CaRs in endocrine (parathyroid and C cells), absorptive, and secretory epithelia (gastrointestinal tract, TAL, distal tubule and IMCD of kidney), bone (osteoclasts), and neurons (SFO and area postrema), that act together to regulate both the steady-state concentration and flux of Ca^{2+} within the extracellular compartment. Modulation of CaR function and expression may provide for important regulatory elements in various states throughout life, including: (1) the transition from fetal to postnatal life, (2) intervals of rapid growth or catabolism requiring the integration of massive fluxes of Ca^{2+} through the extracellular fluid compartment, and (3) preservation of stable Ca^{2+} concentrations in extracellular fluid when abnormalities in sodium, potassium, acid-base, or osmolar balance alter total body water and ion homeostasis. Future research focusing on how CaRs provide for overlapping regulatory pathways in multiple tissues are likely to result in new insights into the prevention and treatment of multiple disorders of water and divalent mineral ion homeostasis.

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