Vasopressin regulation of renal sodium excretion

James D. Stockand[1]

[1]Department of Physiology, University of Texas Health Science Center at San Antonio, San Antonio, Texas, USA

Vasopressin promotes renal water reabsorption decreasing excretion of free water to dilute plasma and lower serum osmolality. We have good understanding of the causes, mechanisms and consequences of this vasopressin-dependent renal water movement. In comparison, vasopressin actions on renal electrolytes including sodium excretion and its consequences have been less well understood. This is so for investigation and discussions of the renal actions of vasopressin are framed primarily around water metabolism rather than any direct effect on salt handling. The fact that water moves in biological systems, to include the mammalian kidney, only by osmosis passively down its concentration gradient is implicit in such discussion but often not overtly addressed. This can cause confusion. Moreover, although vasopressin action on renal sodium excretion via the V2 receptor is critical to water transport, it is masked easily being situational—for instance, dependent on hydration state. It is now clear that an increase in sodium reabsorption along the distal nephron (CNT + CD) mediated by activation of the epithelial Na^+ channel (ENaC) by vasopressin makes an important contribution to maintenance of the axial corticomedullary osmotic gradient necessary for maximal water reabsorption. Thus, we need to modify slightly our understanding of vasopressin and its renal actions to include the idea that while vasopressin decreases free water excretion to dilute plasma, it does this, in part, by promoting sodium reabsorption and consequently decreasing sodium excretion via ENaC activated along the distal nephron.

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Correspondence: *James D. Stockand, Department of Physiology, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Dr, San Antonio, Texas 78229-3900, USA. E-mail: stockand@uthscsa.edu*

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Vasopressin is thought to possess antinatriuretic actions along with its better described antidiuretic actions.[1–8] This is supported by a preponderance of findings from cultured renal cell lines, and renal tissue studied in isolation, including perfused tubules and split-open collecting ducts, demonstrating that vasopressin increases luminal to serosal sodium reabsorption by activating the epithelial Na^+ channel (ENaC).[2,3,7,9–23] This effect should reduce sodium excretion *in vivo*. However, investigation of water and electrolyte handling in animals and humans show vasopressin to have variable effects on net sodium excretion.[4–6,8,24–33] New understanding of the cellular mechanism and systemic consequences of vasopressin action, in combination with reconsideration of earlier findings, reveals why this is so.

VASOPRESSIN SIGNAL TRANSDUCTION

Vasopressin targets two receptor types, V1 and V2.[34–37] Both are seven transmembrane G-protein coupled receptors. The former couples to phospholipase C via $G_{q/11}$ and the latter to $G_{s,}$ ultimately increasing cyclic adenosine monophosphate (cAMP) by stimulating adenylyl cyclase. V1 receptors are most abundant in vascular smooth muscle cells and their stimulation favors contraction. V2 receptors are in epithelial cells, such as principal cells of the distal nephron, and their stimulation increases renal water reabsorption.[34–37] Stimulation of V1 receptors, although not directly involved in control of tubular water and electrolyte transport, increases sodium excretion because of the influences on blood pressure, effective circulating volume, glomerular filtration rate and circulation in the vasa recta system.[24,34,38,39] As illustrated in Figure 1, activation of V2 receptors in the distal nephron by vasopressin stimulates free water reabsorption by promoting cAMP-dependent trafficking of aquaporin 2 water channels to the luminal membrane of principal cells allowing back diffusion of water down its concentration gradient. In addition, as a focus of the current review, vasopressin via V2 receptors also modulates discretionary sodium reabsorption across principal cells mediated by ENaC. This facilitates free water reabsorption by supporting the axial corticomedullary hyperosmotic gradient. Vasopressin via V2 receptors also activates urea transporters, such as UTA1, in the distal nephron to facilitate urea reabsorption and urea recycling, which allows maximization of sodium reabsorption in the thick ascending limb via NKCC2 supporting the axial hyperosmotic gradient drawing water from the distal nephron (reviewed by Sands and Layton[40] and Fenton[41]).

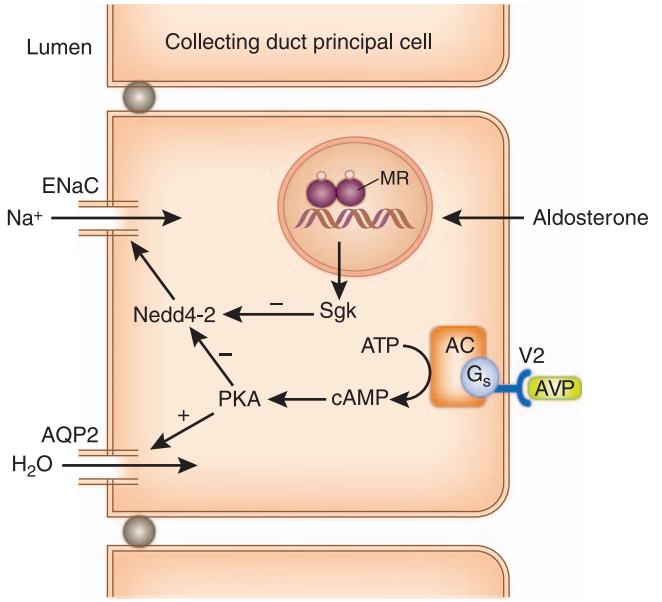

Figure 1 | Vasopressin signal transduction. AC, adenylyl cyclase; AQP2, aquaporin 2; AVP, arginine vasopressin; cAMP, cyclic adenosine monophosphate; ENaC, epithelial Na^+ channel; MR, mineralocorticoid receptor; Nedd4-2, neural precursor cell expressed developmentally downregulated 4-2; Sgk, serum and glucocorticoid-inducible kinase.

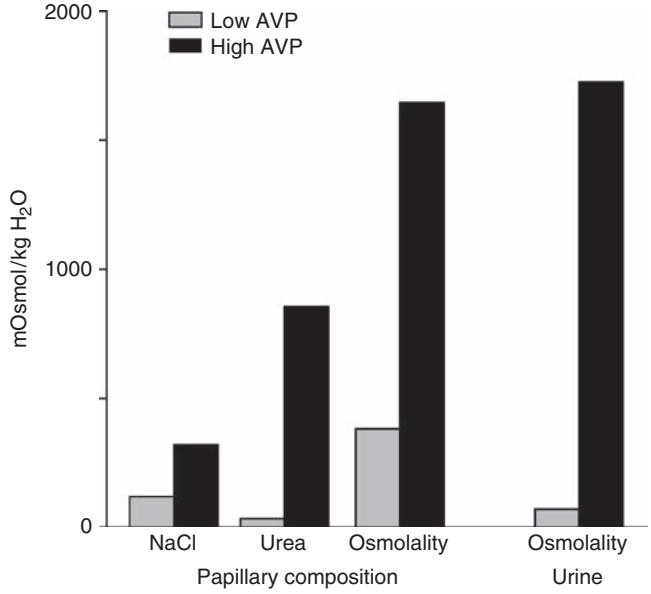

Figure 2 | Vasopressin increases papillary sodium and urea concentrations while concentrating urine. Figure regenerated from data presented originally in Levitin *et al.*[42] AVP, arginine vasopressin.

HISTORICAL PERSPECTIVE

The first clues that vasopressin might decrease renal sodium excretion came from early micropuncture studies and renal tissue fluid sampling circa 1950–60.[42,43] As reproduced in Figure 2, these studies demonstrated that '… antidiuretic hormone enhances the sequestration of sodium in the interstitial fluids of the medulla and papilla'.[42] Importantly, vasopressin increased the absolute amount of sodium per unit of dry solids showing that increases in medullary sodium concentration were independent of effects on water. The converse was also established where decreases in vasopressin lowered the absolute amount of sodium in medullary interstitial fluid. Such findings combined with our understanding of osmotic water movement demonstrate that the effects of vasopressin on sodium transport are primary and contribute to water movement. Also established by pioneering work in renal physiology is that the ability to concentrate urine is dependent on systemic sodium levels with sodium depletion, hyponatremia and removal of plasma sodium with dialysis compromising this ability.[44–48]

SYSTEMIC CONSEQUENCES OF VASOPRESSIN ACTION

Elevated and lower than normal urinary sodium concentrations and excretion have been reported for humans with hyponatremia resulting from upregulated and uncontrolled vasopressin secretion.[24,25,49–51] This is so, as discussed below, because the effects of vasopressin on renal sodium excretion are situational and easily obscured. The renal consequences of vasopressin on sodium excretion include both primary causative actions as well as secondary responses. Moreover, vasopressin controls excretion of free water by manipulating both water permeability and the movement of solute, notably urea and sodium.[34–37] The latter makes control of systemic water and sodium linked to some degree with one in some instances capable of masking or impairing proper regulation of the other. When considering vasopressin action on sodium excretion, it is important to understand that it is only one component of a larger multifactorial homeostatic control system governing systemic sodium balance with most input into this system responding to changes in plasma sodium levels rather than water levels. Thus, vasopressin effects on sodium excretion in the whole animal cannot be considered in isolation and must be viewed in the context of other signals also affecting systemic sodium balance. Similarly, a change in renal sodium excretion in response to vasopressin is just one component of a larger homeostatic control system governing systemic water balance and must be considered in the context of other input, such as the activity of the renin–angiotensin II–aldosterone system (RAAS).

A recent study by Perucca *et al.*[26] that precisely teased apart contribution from specific V1 and V2 receptor agonism and antagonism to renal water and sodium handling in rats demonstrated that vasopressin via V2 receptors decreases renal sodium excretion in addition to decreasing water excretion. As recapitulated in Figure 3, specific V2 agonism acutely decreased sodium excretion in a dose-dependent manner, while promoting free water reabsorption and lowering urea excretion. Antagonism of this receptor results in the opposite response: increased sodium and water excretion and urine dilution. Addition of exogenous vasopressin capable of stimulating both V1 and V2 receptors decreases sodium excretion and promotes free water

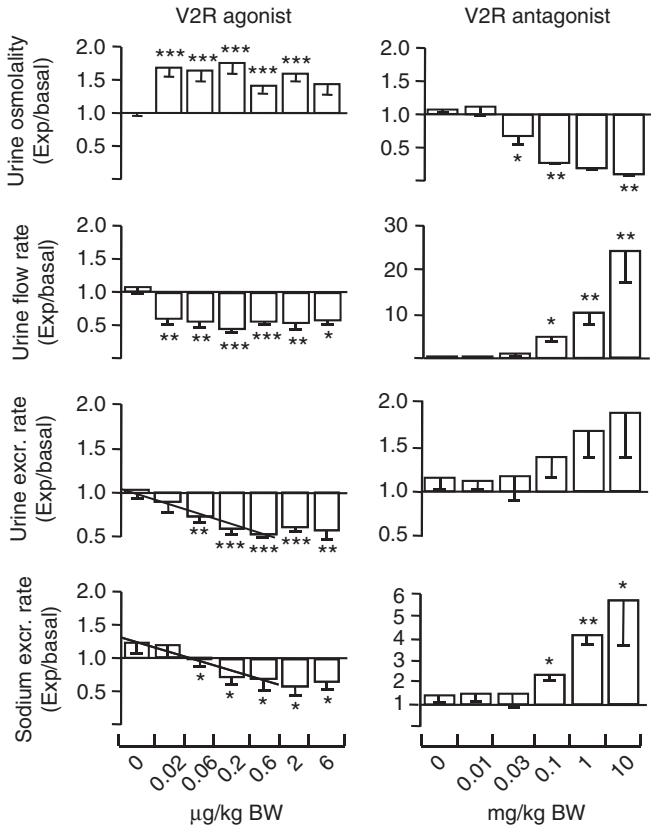

Figure 3 | Vasopressin via V2 receptors (V2Rs) decreases sodium excretion (excr.) while concentrating urine. Figure originally presented in Perucca *et al.*[26] and partially reproduced here. Mean data presented as fold changes under experimental (Exp) conditions as normalized to starting (basal) values in the same rats. Paired *t* test vs basal; $*P<0.05$, $**P<0.01$, $***P<0.001$. BW, body weight.

reabsorption at low doses and at higher doses increases sodium excretion marginalizing free water reabsorption with the latter as proven with specific agonists and antagonists reflecting input from V1 receptors and compensation to obligatory water retention. Thus, activation of V2 favors antidiuresis associated with antinatriuresis, whereas, activation of V1 favors natriuresis. These observations in rat appear to hold for humans, for vasopressin and preferential activation of V2 receptors also reduces sodium excretion in healthy human subjects and those having nephrogenic diabetes insipidus as a result of aquaporin 2 mutation but not those with mutation of the V2 receptor.[4,6]

As demonstrated many years ago in a seminal study from Leaf *et al.*,[24] '… the state of hydration determines the effect of pitressin on electrolyte excretion.' The salient finding in this study performed in humans was that under rigid control of electrolyte and water consumption, exposure to exogenous vasopressin, presented as the posterior pituitary extract, pitressin, only increased sodium excretion secondarily to promoting water retention. This has been reconfirmed recently with the natriuretic response to vasopressin, when observed, brought about principally along the length of the proximal tubule in compensation to volume expansion and increases in blood pressure.[52] Such compensation is capable of obscuring vasopressin-dependent decreases in sodium excretion with secondary increases in sodium excretion temporally following antidiuresis, water retention and development of hyponatremia. This establishes a chronological cause and effect relation: antidiuresis in response to vasopressin is almost immediate with secondary increases in sodium excretion delayed by days.[24] As expected then, secondary increases in sodium excretion end upon correction of volume changes by either promoting water diuresis following cessation of vasopressin treatment or severely restricting water intake.[24,26] These findings and those above, in accord with other observations that endogenous vasopressin released by dehydration does not cause natriuresis,[5,53–55] resolve most of the controversy surrounding the apparent conflicting reports of vasopressin action on renal sodium excretion. The presentation of vasopressin as either antinatriuretic or natriuretic depends on hydration state and specific agonism of either V1 or V2 receptors with vasopressin in the presence of hypervolemia favoring increases in sodium excretion and in the presence of euvolemia and hypovolemia favoring decreases in sodium excretion.[5,6,24,26] As summarized in Figure 4, which was originally presented in,[26] a natriuretic response to vasopressin mediated by V1 receptor stimulation and/or in compensation to obligatory fluid retention can mask direct antinatriuretic actions on renal epithelia mediated by V2 receptors. With such a response, changes to volume predominate lessening the maximal urine concentrating ability of vasopressin shown as declining urine osmolality in the presence of increasing sodium excretion. Decreased sodium excretion in response to vasopressin reflects a primary antinatriuretic response via V2 receptors favoring water reabsorption not obscured by responses to changes in volume. Thus, not unexpectedly, in some instances, homeostatic control of sodium and volume compete with homeostatic control of water and osmolality obscuring direct actions of vasopressin on sodium excretion.

In consideration of the above and the fact that water handling due to osmosis must be linked to some degree with sodium handling, one might predict that the direct effects of vasopressin and other salt retaining signals, such as aldosterone, would be additive with respect to their action on distal nephron sodium reabsorption, and sodium and water excretion. This is exactly what was reported for the actions of pitressin in the absence and presence of exogenous adrenocorticotropic hormone; and what is documented by more recent investigation of aldosterone and vasopressin action on sodium and water excretion, and sodium flux and ENaC activity in whole animal studies, isolated perfused tubules and split-open collecting ducts.[8–11,56]

V2 RECEPTOR BLOCKADE

Another point of contention causing confusion are findings that selective antagonism of V2 receptors acts primarily as an aquaretic having no or only little effect on electrolyte

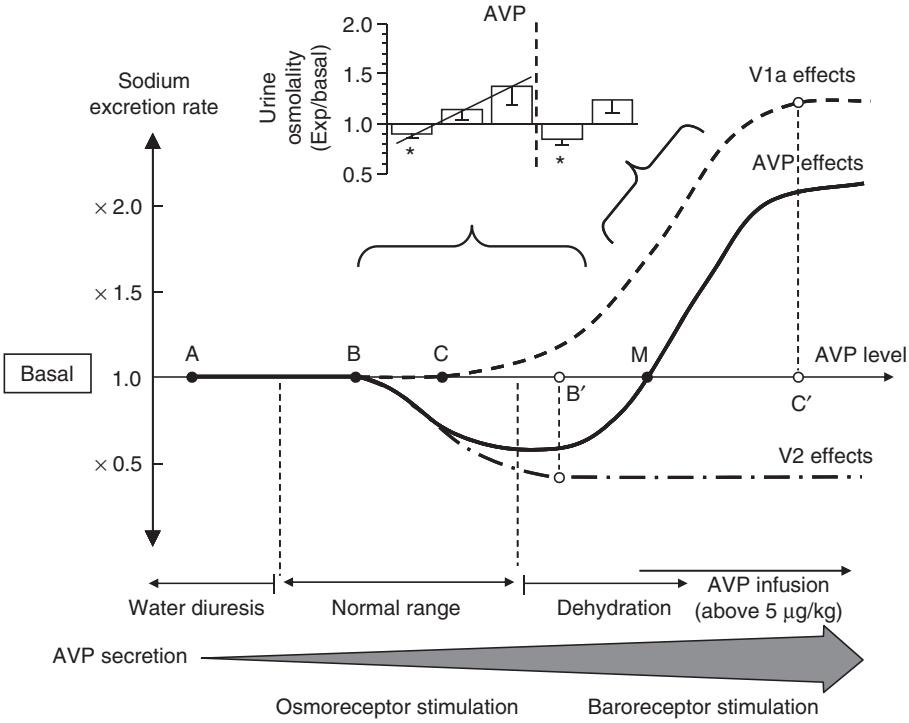

Figure 4 | Schematic representation of the dose-dependent effects of AVP on sodium excretion rate and dissociation of these effects into V2 receptor (V2R)- and V1aR-mediated responses. Figure from Perucca *et al.*[26] The abscissa represents increasing levels of plasma AVP from left (undetectable) to right. A corresponds to the lowest values of AVP, which reduce urine flow rate but not sodium excretion rate. V2R antinatriuretic and V1aR natriuretic effects are depicted as sigmoid curves with different thresholds (B for V2R and C for V1 effects). (B′ and C′) Correspond to the maximum effects depending on each receptor type, respectively. M corresponds to the value of plasma AVP for which the antinatriuretic and the natriuretic effects compensate each other. This value probably fluctuates according to a number of factors influencing the intensity of the responses mediated by each of the two receptor types. Insert showing dose-dependent effects of AVP on urine osmolality also from Perucca *et al.*[26] Dashed line indicates AVP dosage that results in decreases in sodium excretion that favor reabsorption of free water (left) from those resulting in increases in sodium excretion compromising urine concentrating ability. AVP, arginine vasopressin; Exp, experimental.

excretion.[56–58] Restated, the aquaretic actions of V2 antagonism are far larger than their natriuretic effects. This is at variance to the actions of stronger diuretics. The reason for this is that vasopressin influences water movement by affecting both sodium transport and water permeability. Moreover, vasopressin only transiently affects sodium excretion. In the study by Perucca *et al.*,[26] the diuretic but not natriuretic actions of V2 antagonism persisted over a 24 h period. Acute natriuretic actions of V2 antagonism, though, are apparent at earlier time points but wan thereafter as sodium is retained to compensate for marked water (volume) loss. Notably, most studies reporting contrary findings about the effect of vasopressin (or specific V2) antagonism on electrolyte excretion focus on the 24 h or later time point.[59–61] Indeed, careful inspection of these conflicting studies show in agreement marked increases in sodium excretion at earlier time points upon V2 receptor inhibition.[59,61,62] This is consistent with the concept that blocking vasopressin action on renal epithelia during water retention leads to an acute but transient natriuretic response contributing to marginalization of free water reabsorption. This makes sense if compromise of a well-developed axial corticomedullary hyperosmotic gradient favoring free water reabsorption requires only transient increases in sodium excretion. As first suggest in 1962 by Levitin *et al.*,[42] '…the total quantity of sodium necessary to raise the content of sodium of the medulla from that observed during water diuresis to that present during hydropenia is very small.' Following logically, reversal of a state favoring water reabsorption to one favoring water excretion requires only transient increase in sodium excretion.

ENaC ACTIVITY SETS SODIUM REABSORPTION IN THE DISTAL NEPHRON

Studies of the distal nephron in isolation provide direct proof that vasopressin increases sodium reabsorption here. In addition, the cellular mechanism and final effector associated with increases in sodium reabsorption and thus, decreases in excretion now have been unequivocally determined. Like aquaporin 2, ENaC is in the apical membrane of principal cells. Here, ENaC serves as the primary, if not only, gateway for electrogenic sodium reabsorption. Consequently, ENaC activity is limiting for discretionary Na^+ reabsorption in this tissue.[63–67] ENaC is blocked by the K^+-sparing diuretic amiloride with inhibition increasing sodium excretion.[63,68] As the limiting step in Na^+ reabsorption and a target of the

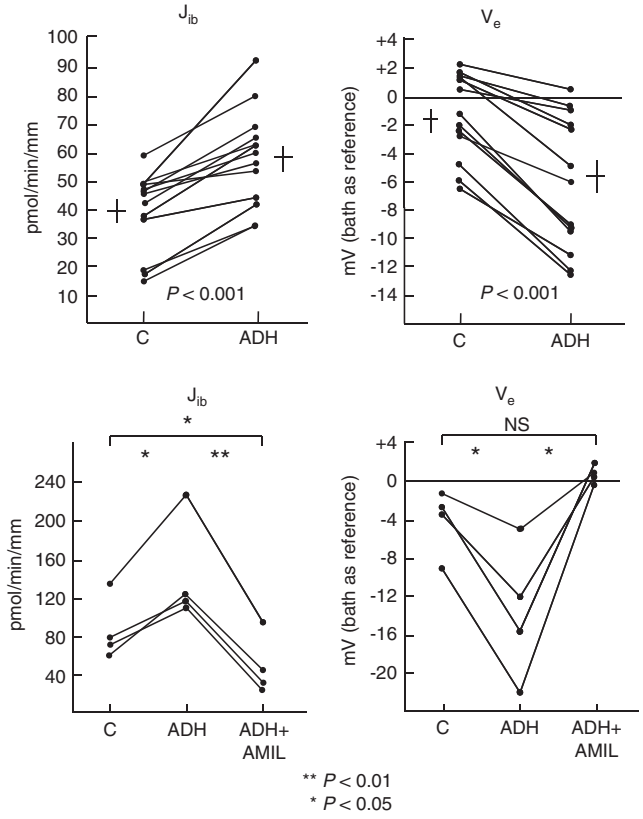

Figure 5 | Vasopressin increases amiloride-sensitive luminal to serosal sodium flux (J_{lb}; left) and transepithelial voltage (V_e; right) in the isolated perfused rat collecting tubule. Figures originally from Reif *et al.*,[10] control (C), antidiuretic hormone (ADH) and ADH + amiloride (AMIL) indicated control plus vasopressin in the absence and presence of AMIL.

adrenal corticosteroid aldosterone, ENaC serves as a critical end effector of RAAS governing plasma Na^+ levels and thus, blood volume and pressure via negative feedback regulation.[65–67] The importance of ENaC and its proper regulation to renal Na^+ handling is clear when considering the inappropriate sodium retention and hypertensive phenotype associated with ENaC gain of function, and the renal sodium wasting associated with loss of function.[66,67,69] Thus, increases and decreases in ENaC activity are causative for changes in renal sodium excretion.

VASOPRESSIN INCREASES ENaC ACTIVITY

Indication that ENaC is involved in a physiologically important vasopressin response first came from a series of detailed studies by the Schafer and Burg laboratories in isolated perfused tubules.[2,3,10–14,70] As recapitulated in Figure 5, vasopressin increases the potential difference across the isolated collecting duct consistent with increasing active ion transport.[2,3,10–14,70] The increase in potential difference upon exposure to vasopressin is caused by a dose-dependent change in net sodium flux (sodium reabsorption) from the luminal fluid to serosal bath fluid with the lumen becoming more hyperpolarized.[3,10–14,70] Moreover, vasopressin-dependent increases in sodium flux and transepithelial voltage changes are often associated with decreases in transepithelial resistance (and increases in conductance) consistent with activation of apical sodium channels.[2,11,70] The inhibitor of ENaC, amiloride, abolishes this sodium flux, and changes in voltage and resistance pointing to flux through this channel as the limiting step in vasopressin-dependent sodium reabsorption.[10,11] Concomitant with increases in net lumen to serosal sodium flux are increases in water permeability and water reabsorption from the luminal to serosal fluid.[12,13]

Although the above studies strongly supported vasopressin activation of ENaC in the mammalian collecting duct, direct evidence for this was obtained only recently. We used patch clamp electrophysiology to specifically test whether vasopressin increases ENaC activity in the isolated, split-open murine collecting duct. As represented in Figure 6, vasopressin increases ENaC activity by increasing channel open probability (P_o) and the number (N) of channels in the apical plasma membrane.[9] ENaC in tubules isolated from water restricted animals also had elevated activity in response to increases in endogenous vasopressin.[9] Inhibition of cAMP signaling compromised increases in ENaC activity in response to vasopressin and water restriction. Vasopressin via cAMP signaling is known to promote trafficking and insertion of ENaC into the apical membrane of principal cells.[18,71–74]

Vasopressin, in addition, has a robust effect on amiloride-sensitive short-circuit current across cultured epithelial cells, and ENaC activity in immortalized renal epithelial cells.[17–23] cAMP has similar actions on ENaC in cultured cells and increases sodium flux and the Na^+ conductance of the luminal membrane in isolated tubules.[11,18,19,70,75] Such observations demonstrate that vasopressin uses V2 receptors coupled to G_s and stimulation of adenylyl cyclase and production of cAMP as a common signaling pathway to increase both ENaC and aquaporin 2 activity. At a cellular level, the previous increases sodium reabsorption to strengthen osmotic draw of water and the latter increases water permeability.

VASOPRESSIN AND ALDOSTERONE ACT IN A SYNERGISTIC MANNER

As shown in Figure 7, ENaC is most active in tubules that come from animals deprived of both water and Na^+ or treated with both aldosterone and vasopressin.[9–11,13,14] This is consistent with findings that vasopressin has its greatest effect to decrease sodium excretion in hypovolemic states.[5,26,50] ENaC, though, also is active during conditions, such as high dietary sodium intake combined with water restriction, that promote water but not necessarily sodium conservation supporting the idea that vasopressin has a quantitatively important action on ENaC during water reabsorption uncoupled from the role played by the channel in sodium balance. This action is to decrease sodium excretion to facilitate water reabsorption. Consistent with this, mice with tissue specific deletion of the mineralocorticoid receptor in

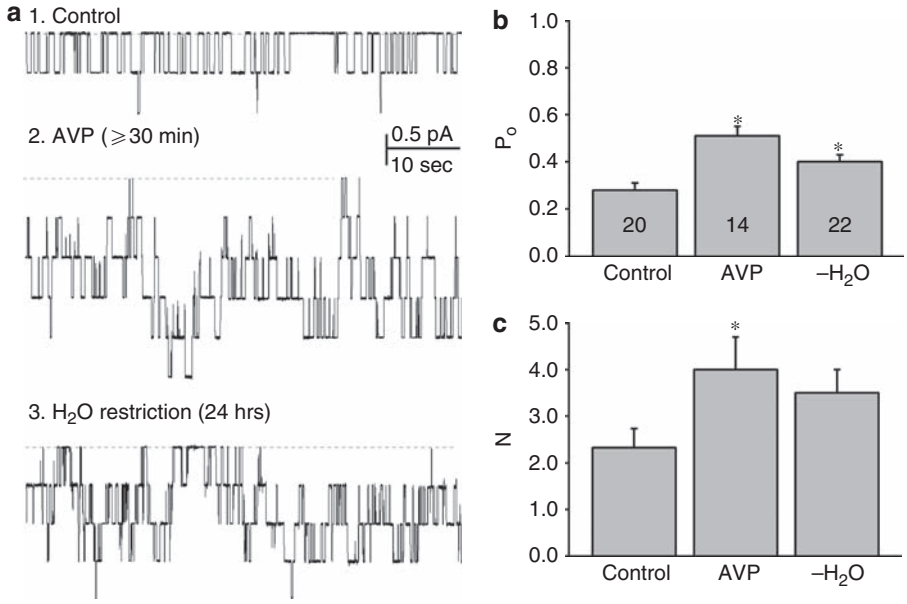

Figure 6 | Vasopressin and water restriction increase epithelial Na^+ channel (ENaC) activity (N and P_o) in the isolated, split-open mouse collecting duct as assessed with patch clamp analysis. Figure originally from Bugaj *et al.*[9] (**a**) Raw single channel current traces for cell-attached patches. (**b** and **c**) Summary data. *$P<0.05$ vs control. AVP, arginine vasopressin; PO, open probability.

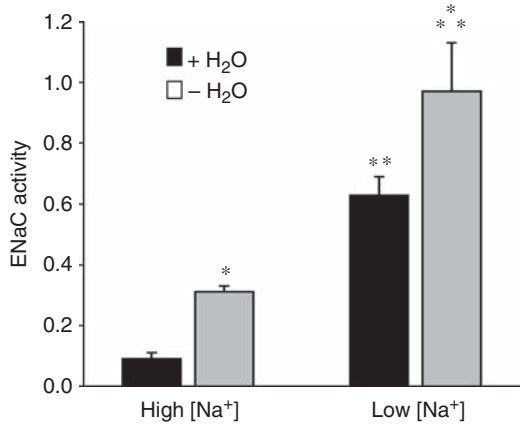

Figure 7 | Sodium and water retaining states act in synergy to increase epithelial NaC (ENaC) activity. Data originally from Bugaj *et al.*[9] High and low [Na^+] indicate isolated split-open collecting ducts from mice maintained with 2% [Na^+] and nominally sodium-free feeding regimens, without (black) and with (gray) additional water deprivation. * vs $+H_2O$, ** vs high [Na^+].

principal cells show increased water excretion when stressed with low salt.[76] Similarly, urinary concentrating defects are known to result from adrenal insufficiency emphasizing the importance of the additive effects vasopressin and aldosterone have on ENaC to decrease sodium excretion and support water reabsorption.[77]

At a cellular level, convergence arises, in part, from vasopressin via cAMP/protein kinase A and aldosterone via serum and glucocorticoid-inducible kinase relieving negative regulation of ENaC by phosphorylating and inhibiting the negative regulator neural precursor cell expressed developmentally downregulated 4-2.[78] Neural precursor cell expressed developmentally downregulated 4-2 is an ubiquitin ligase that promotes removal of ENaC from the plasma membrane.

CONCLUSION

It is important to remember that, although vasopressin actions on net sodium excretion by the kidney are situational and easily obscured when placed in the context of whole animal responses, nephron segments expressing the V2 receptor respond to vasopressin by increasing sodium reabsorption in support of an antidiuretic response. Stated another way, the causative primary actions of vasopressin on ENaC and thus, distal nephron sodium transport always supports antidiuresis by decreasing sodium excretion but this may be obscured by secondary actions or the actions of other hormones or factors influencing sodium balance rather than water homeostasis.

DISCLOSURE

The author declares no competing interests.

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