Vasopressin-V2 Receptor Stimulation Reduces Sodium Excretion in Healthy Humans

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In addition to its effect on water permeability, vasopressin, through its V2 receptors (AVPR2), stimulates Na reabsorption in the collecting duct by increasing the activity of the amiloride-sensitive sodium channel ENaC. This study evaluated whether dDAVP (a potent AVPR2 agonist) reduces sodium excretion in healthy humans (n = 6) and in patients with central (C; n = 2) or nephrogenic (N) diabetes insipidus (DI) as a result of mutations of either the aquaporin 2 gene (AQP2; n = 3) or AVPR2 (n = 10). dDAVP was infused intravenously (0.3 μg/kg body wt in 20 min), and urine was collected for 60 min before (basal) and 150 min after the infusion. dDAVP markedly reduced both urine flow rate and sodium excretion in healthy individuals. A reduction in sodium excretion was also observed in CDI and NDI-AQP2 patients but not in NDI-AVPR2 patients. The magnitude of the fall in sodium excretion correlated with the rise in urine osmolality and the fall in urine output but not with the simultaneously observed fall in mean BP. These results suggest that the dDAVP-induced antinatriuresis is due to a direct V2 receptor–dependent stimulation of sodium reabsorption in the collecting duct and is not secondary to a hemodynamic effect. In conclusion, this study reveals a potent V2-dependent antinatriuretic effect of vasopressin in humans. The possibility that an inappropriate stimulation of ENaC by vasopressin might lead to significant sodium retention in chronic situations remains to be determined.

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Inappropriate retention of sodium and water by the kidney is thought to be a key factor in several forms of hypertension. Thus, it is important to identify factors that may favor excessive sodium reabsorption by the kidney. Vasopressin is known to promote water conservation by increasing the permeability to water of the collecting ducts (CD), thus allowing osmotically driven water reabsorption along these ducts when dilute distal tubular fluid enters them in the cortex and later when they traverse the hyperosmotic medulla. However, the effect of vasopressin on sodium excretion is less clear. A large number of studies have shown that vasopressin infusion increases sodium excretion *in vivo* in rats, dogs, and sheep (*e.g.*, references 1–4). This natriuretic effect is difficult to reconcile with the fact that, *in vitro*, vasopressin stimulates sodium reabsorption in the isolated perfused CD (5,6), in the amphibian bladder (a tissue that shares a number of similarities with the mammalian CD) (7), and in several cell lines issued from these two tissues (8–10). This effect on sodium reabsorption is inhibitable by amiloride and has been shown to result from activation of the endothelial sodium channel ENaC (11). Moreover, chronic elevation of vasopressin or infusion of its V2 agonist dDAVP (12) has been shown to increase the abundance of mRNA (13) and protein (14) of the β and γ subunits of ENaC and to enhance markedly the subsequent functional response (water and sodium reabsorption) to exogenous dDAVP *ex vivo* (13). Taken together, these *in vitro* and *ex vivo* results suggest that vasopressin should be antinatriuretic *in vivo*. Because the CD is the last portion of the nephron traveled through by urine before exiting the kidney, no rapid compensatory reduction can occur downstream after an increase in vasopressin-induced sodium reabsorption in this segment.

Actually, a few studies suggest that vasopressin might be antinatriuretic or might retard sodium excretion. (*1*) Chouk-roun *et al.* showed that a sodium load was excreted more slowly in healthy subjects when they drank only small amounts of water throughout the study than when they received a much higher sustained hydration (15). (*2*) In the isolated erythrocyte-perfused rat kidney, dDAVP was shown to reduce in parallel urine output and sodium excretion (16). (*3*) In healthy individuals who performed their normal activities, sodium excretion was independent of urine flow rate as long as this flow rate remained above 1 ml/min, but with further reduction in urine output, sodium excretion decreased in parallel with urine flow rate, an observation suggesting that an increase in sodium reabsorption occurred when vasopressin levels rose above a certain threshold (17). That an effect of sodium reabsorption may require a higher level of vasopressin than the effect on water permeability is also apparent in studies of isolated perfused CD (data for water permeability and sodium flux [18] reanalyzed in reference 19). The well-established effect of vasopressin or V2 receptor agonists on ENaC-mediated sodium transport *in vitro* prompted us to evaluate the effects of the

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vasopressin V2 receptor agonist dDAVP on water and solute excretion in healthy individuals as well as in patients with various forms of diabetes insipidus (DI).

Materials and Methods

In previous studies at the Hôpital du Sacré-Cœur (Montreal), healthy individuals and patients with DI had been submitted to an acute dDAVP infusion to evaluate the influence of this drug on hemodynamics, coagulation factors, and plasma cAMP (20–22). These studies were approved by the institutional ethical review committee, and all participants gave written informed consent. The procedures followed were in accordance with institutional guidelines. In these studies, urine had been collected and the concentration of the main urinary solutes had been measured, but these data had not been reported in the initial articles. Because vasopressin is now known to stimulate ENaC-mediated sodium transport in the CD, we decided to reanalyze this unpublished data in an attempt to evaluate the effects of dDAVP on sodium excretion in humans.

Subjects included six healthy men and women and 15 patients with DI. Table 1 shows the gender, age, and body weight of these subjects. Two patients had central DI (CDI), and 13 had nephrogenic DI (NDI) as a result of mutations of either the vasopressin V2 receptor gene (NDI-AVPR2; $n = 10$) or the aquaporin 2 gene (NDI-AQP2; $n = 3$; see the mutations involved and additional information in reference 23). CDI patients stopped their dDAVP treatment during 24 h and NDI patients their hydrochlorothiazide medication during 3 d before the study.

As already described (21,22), after an overnight fast, the subjects underwent an intravenous infusion of dDAVP, 0.3 μg/kg body wt, over 20 min, and urine was collected by spontaneous voiding every 30 min for 60 min before and 180 min after the beginning of dDAVP infusion. Subjects did not receive any water load before the study, and no fluid or food was allowed throughout the study. Urine volume and osmolality (U_{osm}) and urinary creatinine, sodium, potassium, and urea concentrations were measured. Urinary flow rate (V = water excretion) and excretion of the different solutes were calculated. In four of the six healthy subjects, the same protocol was repeated with the same timing, during a sham test involving infusion of isotonic saline instead of dDAVP.

Statistical Analyses

Results are expressed as means ± SEM. The basal period is the mean of the two half-hour periods preceding the dDAVP infusion. The dDAVP period is the mean of the 30- to 60-, 60- to 90-, and 90- to 120-min period after the start of the dDAVP infusion. The first 30 min of dDAVP infusion are shown in the figures but were not included in the means used for the statistical analysis as they represent a transitional situation. The later periods after 120 min were also not included in the means to exclude the possible influence of indirect factors coming into play in response to the initial dDAVP-induced changes. The influence of dDAVP between the basal and dDAVP periods was evaluated by paired t test. The difference in the natriuretic response to dDAVP between DI patients with functional or with nonfunctional V2 receptor was evaluated by t test. $P < 0.05$ was considered significant.

Results

The time course of changes observed in healthy subjects and DI patients after dDAVP infusion is shown in Figures 1 and 2, respectively. In healthy subjects, dDAVP induced an abrupt and marked decline in urine flow rate (by 70%) and a modest progressive rise in urine osmolality. Note that urine was already relatively concentrated during the basal period (U_{osm} = 686 ± 112 mOsm/kg H_2O), a normal situation in healthy individuals. Because the rise in U_{osm} did not compensate for the marked fall in urine flow rate, osmolar excretion fell by 52%. The fall in osmolar excretion was mostly accounted for by a significant abrupt fall in sodium and urea excretions (−69 and −57%, respectively). In contrast, potassium excretion exhibited only a modest and progressive decline (−21%). Creatinine excretion declined slightly (but not significantly) during the first period after dDAVP administration and remained stable thereafter at 10 to 15% below basal level (Figure 1). In contrast to dDAVP infusion, infusion of isotonic saline in four healthy time-control subjects did not induce any significant change in either fluid or solute excretions (Table 2).

The average values observed during the basal and dDAVP periods, as well as the dDAVP/basal ratios for all groups, are shown in Table 2. In CDI patients, dDAVP induced a significant decline in urine flow rate and rise in U_{osm} that reached a plateau after 90 min. In contrast, dDAVP failed to induce a rise in U_{osm} in patients with NDI-AQP2 and even induced a small but significant increase in those with NDI-AVPR2 (from 106 ± 19 to 133 ± 15 mOsm/kg H_2O; $P = 0.01$; Figure 2). Surprising, urine flow rate fell by 36% during the first 60 min in patients with NDI-AQP2 (Figure 2) but did not change in those with NDI-AVPR2.

With respect to sodium, CDI patients exhibited an abrupt fall in sodium excretion that stabilized at approximately 40% of the

Table 1. Description of the different groups of subjects[a]

Subjects	Infusion	n	Gender	Age (yr)	Body Weight (kg)
Healthy	Isotonic saline	4	2 M, 2 F	27 ± 4	66 ± 7
Healthy	dDAVP	6	3 M, 3 F	31 ± 4	67 ± 6
CDI	dDAVP	2	1 M/1 F	12/16	43/61
NDI-AQP2	dDAVP	3	2 M/1 F	10/18/21	29/56/53
NDI-AVPR2	dDAVP	10	10 M	35 ± 5	77 ± 3

[a]Means ± SEM for healthy and NDI-AVPR2 groups. Individual values for CDI and NDI-AQP2 subjects. The four healthy subjects who received an infusion of isotonic saline belong to the group of six who received dDAVP on a different day. CDI, central diabetes insipidus; NDI, nephrogenic diabetes insipidus; AQP2, aquaporin 2 gene; AVPR2, vasopressin V2 receptor gene.

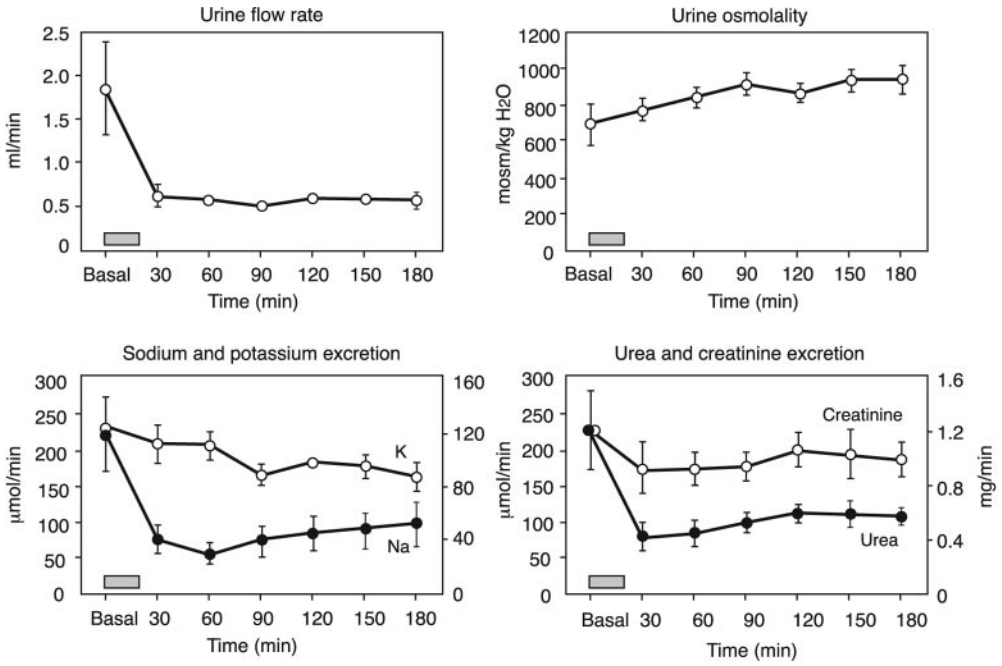

Figure 1. Time course of changes in urine flow rate and osmolality and in sodium, potassium, urea, and creatinine excretion before (basal) and after dDAVP infusion (0 to 20 min; ▨) in six healthy individuals. Means ± SEM.

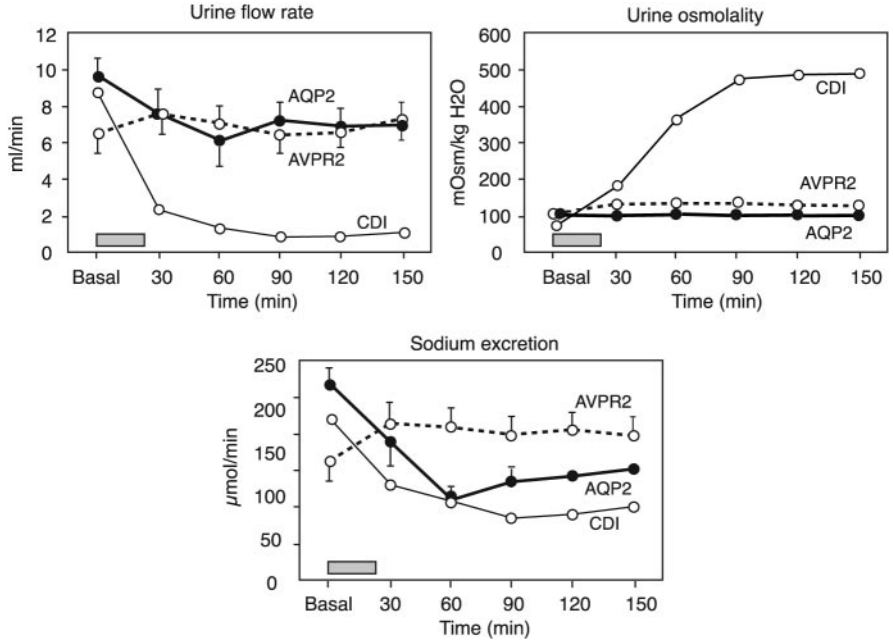

Figure 2. Time course of changes in urine flow rate and osmolality and in sodium excretion before (basal) and after dDAVP infusion (▨) in patients with diabetes insipidus (DI). Means ± SEM. CDI, central DI ($n = 2$); AQP2, nephrogenic DI as a result of mutations of the AQP2 gene ($n = 3$); AVPR2, nephrogenic DI as a result of mutations of the vasopressin V2 receptor gene ($n = 10$).

basal value after 90 min. It is interesting that sodium excretion also fell abruptly, by approximately 40%, during the first 60 min in patients with NDI-AQP2 but not in those with NDI-AVPR2 (Figure 2, Table 2). Because dDAVP is a V2 receptor agonist, it was interesting to compare DI patients with intact V2 receptors ($n = 2$ CDI + 3 NDI-AQP2) with DI patients with nonfunctional V2 receptors ($n = 10$ NDI-AVPR2). Sodium excretion decreased markedly in the former (-107 ± 19 µmol/min; $P = 0.005$) but increased modestly in the latter (33 ± 22 µmol/min; NS). The difference between the two groups is highly significant ($P = 0.002$). Note that the small rise in sodium excretion (NS) that occurred in NDI-AVPR2 patients may be a time-dependent effect because a similar rise was also observed in healthy subjects who received saline infusion (Table 2).

Table 2. Excretion of water (urine flow rate), of osmoles, and of the different solutes before (basal) and 30 to 120 min after infusion of dDAVP (or isotonic saline) in the different groups of subjects[a]

	Water Excretion (ml/min)	Osmolar Excretion (μOsm/min)	Sodium Excretion (μmol/min)	Potassium Excretion (μmol/min)	Urea Excretion (μmol/min)	Creatinine Excretion (mg/min)
Healthy subjects ($n = 4$)						
basal	1.91 ± 0.65	849 ± 54	197 ± 7	86 ± 18	ND	ND
isotonic saline (basal2)	2.13 ± 0.31	1053 ± 143	271 ± 34	101 ± 15	ND	ND
basal2/basal	1.12	1.24	1.38	1.17		
Healthy subjects ($n = 6$)						
basal	1.85 ± 0.53	1009 ± 185	223 ± 51	78 ± 13	225 ± 54	1.21 ± 0.24
dDAVP	0.56 ± 0.06[b]	477 ± 55[c]	70 ± 19[d]	62 ± 3	97 ± 13[c]	0.98 ± 0.11
dDAVP/basal	0.30	0.47	0.31	0.79	0.43	0.81
CDI ($n = 2$)						
basal	8.83	648	184	32.6	216	0.71
dDAVP	1.01	434	79	44.4	126	0.70
dDAVP/basal	0.11	0.67	0.43	1.36	0.58	0.99
NDI-AQP2 ($n = 3$)						
basal	9.70 ± 0.92	977 ± 50	224 ± 17	125 ± 17	279 ± 46	0.88 ± 0.25
dDAVP	6.97 ± 0.97	721 ± 71	115 ± 13	136 ± 10	214 ± 56	0.76 ± 0.25
dDAVP/basal	0.72	0.74	0.51	1.09	0.77	0.86
NDI-AVPR2 ($n = 10$)						
basal	6.51 ± 0.97	592 ± 85	137 ± 25	41.1 ± 4.7	235 ± 46	1.00 ± 0.11
dDAVP	6.76 ± 0.99	798 ± 64[b]	171 ± 16	61.6 ± 9.0[c]	326 ± 38	1.14 ± 0.06
dDAVP/basal	1.04	1.35	1.25	1.50	1.39	1.14

[a]Water and solute excretion under "dDAVP" is the mean of three half-hour periods after the start of the dDAVP infusion (30 to 60, 60 to 90, and 90 to 120 min). The four healthy subjects who received an infusion of isotonic saline belong to the group of six who received dDAVP on a different day. Data are means ± SEM. ND, not determined.

Paired *t* test, dDAVP *versus* basal: [b]$P = 0.06$; [c]$P < 0.05$; [d]$P < 0.01$.

Figure 3 displays water and sodium excretion as a function of time, expressed as percentage of basal values. This graphical representation allows comparison of the relative amplitude of changes. In healthy individuals, the fall in urine flow rate and that in sodium excretion are almost identical in amplitude, suggesting that sodium and water were reabsorbed simultaneously and in equal proportions in response to dDAVP. In patients with CDI, the fall in urine flow rate exceeded that in sodium excretion. This may be explained by the fact that urine was very dilute in these patients at the time of dDAVP infusion. Thus, large amounts of water were available for reabsorption as soon as water permeability of the CD epithelium was increased by dDAVP. Thus, a fraction of the reabsorbed water was "solute-free water," whereas another fraction was associated with sodium, as in healthy patients. In patients with NDI-AQP2, the fall in sodium excretion exceeded that in urine output, suggesting that sodium was reabsorbed in excess to water. Finally, in NDI-AVPR2 patients, sodium excretion rose by approximately 30%, somewhat more than did water excretion.

It is interesting to note that the magnitude of the dDAVP-induced reduction in sodium excretion was almost similar in CDI and NDI-AQP2 patients and only slightly less than in healthy subjects; the differences between basal and dDAVP periods (calculated from data shown in Table 2) were 105 and 109 μmol/min in CDI and NDI-AQP2 patients, respectively, and 153 μmol/min in healthy subjects. Surprising, patients with these two forms of DI exhibited a much larger fall in urine flow rate after dDAVP than healthy subjects. The fall in urine flow rate (calculated from data shown in Table 2) amounted to 7.82 ml/min in CDI patients and 2.73 ml/min in NDI-AQP2 patients *versus* only 1.29 ml/min in healthy subjects. One should keep in mind, however, that healthy subjects already had a much lower urine flow rate than the two other groups at the start of the experiment.

In the three groups that reduced their sodium excretion in response to dDAVP (all groups except NDI-AVPR2), we looked for possible correlations between the changes in sodium excretion and those in water excretion (*i.e.*, in urine flow rate). Differences between each of the 30-min post-dDAVP periods (from 0 to 150 min) and the basal period were considered. Figure 4 reveals highly significant positive correlations between sodium and water movements in the three groups. Note that the regression lines for healthy subjects and for patients with NDI-AQP2 almost pass through zero for both axes. However, the slope is significantly steeper for the NDI patients than for healthy subjects. In CDI patients, a significant fall in water reabsorption occurred before any change in sodium reabsorption was observed (shown as "W" in Figure 4).

In their previously published papers, Bichet *et al.* (20,22) reported that dDAVP not only exerted a potent antidiuretic

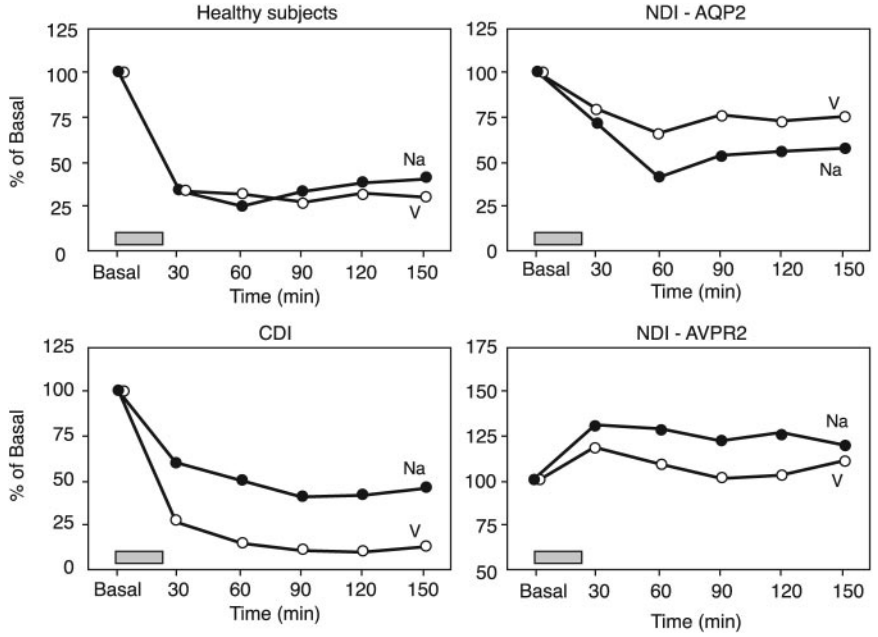

Figure 3. Time course of changes in urine flow rate and sodium excretion in healthy subjects and the three groups of patients with DI, expressed relative to the basal values.

action but also induced a significant 10 to 15% fall in BP in normal subjects and in DI patients but not in those with defective V2 receptors (see Figure 1 in reference 23). This led us to evaluate the relationships between the antinatriuretic effect of dDAVP and its hemodynamic and/or antidiuretic effects in healthy individuals by looking for possible correlations between the changes in sodium excretion and those in BP or in urine osmolality. Differences between each of the 30-min post-dDAVP periods (from 0 to 150 min) and the basal period were considered. As can be seen in Figure 5, top, a highly significant correlation was observed between the fall in sodium excretion and the rise in urine osmolality after dDAVP infusion. In contrast, no significant correlation was observed between the fall in sodium excretion and that in BP (Figure 5, bottom). Moreover,

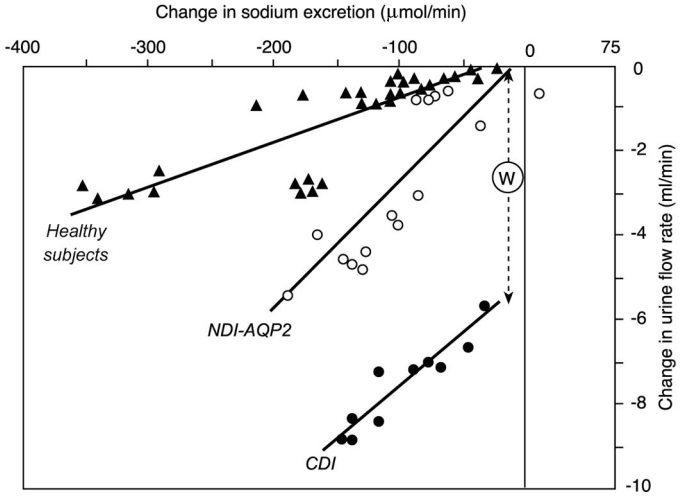

Figure 4. Relationship between the changes in sodium excretion and the changes in urine flow rate (water excretion) in healthy subjects and patients with CDI or NDI-AQP2. W, solute-independent water reabsorption. Correlation coefficients (r) are 0.823 for healthy subjects, 0.805 for NDI-AQP2 patients, and 0.940 for CDI patients ($P < 0.0001$ for each).

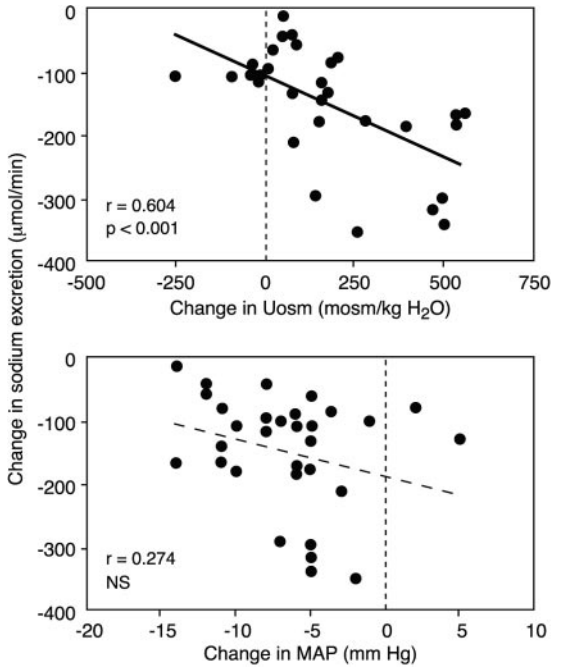

Figure 5. Relationship between the changes in sodium excretion and the changes in urine osmolality (top) or in mean arterial BP (bottom) in healthy subjects. Correlation coefficients and probability of statistical significance are indicated.

the reduction in sodium excretion was even larger when the fall in BP was less intense, contrary to what would be expected if the fall in BP was inducing the fall in sodium excretion. Finally, a marked fall in sodium excretion (by approximately 200 μmol/min) was present even without any change in BP (for Δmean arterial pressure = 0).

Discussion

The results obtained in this study reveal, for the first time to our knowledge, a potent antinatriuretic effect of the vasopressin V2 agonist dDAVP, associated with its well-known antidiuretic action. This effect is solute-selective because potassium and creatinine excretion did not change significantly in response to dDAVP. The antinatriuretic effect of dDAVP also occurred in all patients with intact V2 receptors (CDI and NDI-AQP2) but not in those with defective V2 receptors. These results strongly suggest that vasopressin, through its V2 receptor–mediated actions, favored a more avid sodium reabsorption by the kidney.

Importantly, healthy individuals and DI patients in this study were not submitted to water loading or to any special experimental maneuver before the dDAVP infusion. Healthy individuals exhibited normal urine osmolality (close to 700 mOsm/kg H_2O). This means that endogenous vasopressin was allowing osmotic equilibration of the urine with the surrounding interstitium along the cortical and medullary CD even before dDAVP was infused.

Along with the fall in urine flow rate and sodium excretion, a marked fall in urea excretion was observed in healthy individuals. It is easily explained by the reduction in urine flow rate, known to favor passive urea reabsorption along the whole CD, and by the direct effect of antidiuretic hormone on urea permeability of the terminal CD (24). The relative constancy of potassium excretion is probably due to a balance between two opposite effects, increased reabsorption favored by the reduction in urine flow rate, and stimulation of potassium secretion by antidiuretic hormone (25). The modest (NS) fall in creatinine excretion might suggest a fall in GFR accompanying the previously described fall in BP (20,22). However, renal blood flow and GFR are known to be autoregulated and, thus, to stay stable in the face of changes in BP. Moreover, if GFR had declined for the 3 h post-dDAVP, then a progressive return of creatinine excretion to basal level would have been expected as a result of a rise in plasma creatinine concentration. In the present case, the fall in creatinine excretion was sustained for 3 h. Actually, creatinine is not a perfect marker of GFR. It is known to exhibit net secretion at high urine flow rates and net reabsorption at low urine flow rates (26,27). Thus, the small sustained fall in creatinine excretion could be due to some reabsorption in the distal nephron favored by the longer contact time associated with the reduction in urine flow rate. However, in the absence of GFR measurements in this study, a small fall in GFR cannot be ruled out.

Although the mechanism by which dDAVP influences water, urea, and potassium excretions seems straightforward, the mechanism leading to increase sodium reabsorption may be more complex. Vasopressin binding to V2 receptors in principal cells of the CD not only increases the permeability to water of the luminal membrane through its action on AQP2 but also increases sodium reabsorption by stimulating the activity of the epithelial sodium channel ENaC (5,28). Thus, it is logical to assume that V2 actions of vasopressin on the CD should reduce sodium excretion by a direct effect, as is discussed further below. However, a number of studies have now well documented the existence of extrarenal V2 receptors, probably located on the vascular endothelium (29). The stimulation of these receptors by V2 agonists results in a significant vasodilation (30,31) that probably accounts for the fall in BP observed in this study (20,22).

A rise in BP is known to increase sodium excretion by reducing sodium reabsorption in the kidney, the so-called "pressure-natriuresis" phenomenon. The fall in BP observed in this study thus could have contributed to the apparent antinatriuretic effect of dDAVP. However, no correlation was found between the (experimental − basal) change in sodium excretion and that in BP, whereas a highly significant correlation was observed between the change in sodium excretion and that in urine osmolality (Figure 5). Although no causality link can be formally deduced from such *a posteriori* analyses, these results support the hypothesis that the influence of dDAVP on sodium excretion, if any, occurred through its antidiuretic action on the kidney rather than through its peripheral action on the vasculature.

It is not possible to evaluate in this retrospective study whether a fall in GFR might have contributed to the fall in sodium excretion seen after dDAVP administration. However, as recalled above, if GFR had declined, then autoregulation would have returned it promptly to basal level. Even in the face of a reduction in GFR, the tubuloglomerular balance should have contributed to adapt sodium reabsorption to the actual level of GFR, in the absence of other perturbations. Pressure-induced perturbations in GFR and sodium excretion thus should be only transient. Because sodium excretion remained low for 3 h, along with the progressive dDAVP-induced rise in U_{osm}, it may be assumed that it was not a consequence of a fall in GFR.

Within the kidney, vasopressin is known to stimulate sodium reabsorption in two nephron segments, the thick ascending limb (TAL) and the CD. However, the effect on the TAL seems to be restricted to rodents (19) and to be part of a series of adaptations of the rodent kidney that improve its urine-concentrating ability (32). Vasopressin does not stimulate adenylate cyclase in the human TAL (which, however, is sensitive to other hormones) (33,34). Thus, it may be assumed that, in this investigation, the change in sodium excretion did not depend on an increase in sodium reabsorption in the TAL.

The antinatriuretic effect of dDAVP thus is most likely due to the stimulation of ENaC-mediated sodium reabsorption in the CD. This stimulation has been well described *in vitro* (6,9,13), but its functional consequences *in vivo* have not yet been documented to our knowledge. Actually, even a modest stimulation of sodium reabsorption in the CD should lead to a significant reduction in sodium excretion. Assuming that 5% of the filtered load of sodium is reabsorbed in the CD, leaving 1% to

be excreted in the urine, a stimulation of sodium reabsorption by only 10% (*i.e.*, increasing reabsorption from 5.0 to 5.5%) should reduce sodium excretion by half (from 1.0 to 0.5%).

As already explained, an additional sodium reabsorption in the CD will result in an additional water reabsorption as a result of the osmotic drive generated by the efflux of sodium from the CD fluid (13,35). This indeed is what was observed after dDAVP infusion in all subjects with functional V2 receptors. Highly significant positive correlations were observed between sodium and water movements in the three groups that reduced their sodium excretion in response to dDAVP infusion (Figure 4). These correlations suggest that dDAVP stimulated ENaC-mediated sodium reabsorption from the CD and that this ion movement from lumen to blood drove an equivalent amount of water from the CD.

In subjects with defective AQP2, vasopressin action on V2 receptors and signal transduction through cAMP are not impaired. Thus, sodium reabsorption was probably stimulated by dDAVP as in normal individuals, accounting for the fall in sodium excretion. Unexpected, relatively large amounts of water were reabsorbed along with sodium in these patients despite their genetically impaired vasopressin-regulated water channel. It may be assumed either that the mutations seen in these patients did not completely impair the water transporting capacity of AQP2 or that water was reabsorbed by an AQP2-independent pathway when the osmotic driving force was increased by dDAVP-mediated sodium reabsorption. It is interesting that a significant, osmotically driven water reabsorption has been observed in a cell line in which sodium transport is stimulated by vasopressin but in which no known aquaporins are expressed (10). Further studies are required to determine the mechanism of this unexpected water reabsorption.

In CDI patients, a strong drive for water reabsorption from dilute urine must exist along the CD that is surrounded by an iso-osmotic interstitium in the cortex and by a hyperosmotic interstitium in the medulla (osmotic pressure is elevated to some extent in the medulla, even in DI [36], as a result of accumulation of sodium chloride). Thus, as soon as dDAVP permeates the CD to water, a large mass of water can be reabsorbed. However, even in this setting, an additional amount of water was further reabsorbed in association with sodium, as shown by the positive correlation between sodium and water movements in these patients (Figure 4).

These results showing potent antinatriuretic effects of vasopressin V2 agonism in the human kidney seem to be contradictory to a number of previous studies that reported large natriuretic effects of the native hormone vasopressin. Differences in the experimental conditions and/or in the agonists used may explain these divergent results. With regard to differences in experimental conditions, subjects in this study did not undergo any water diuresis before dDAVP administration. Thus, they produced hyperosmotic urine, and the flow through their CD was relatively small. In this setting, V2 agonism probably stimulates sodium reabsorption in cells that are already highly permeable to water. Consequently, parallel reductions in water and sodium excretion are observed. In contrast, in protocols in which a water diuresis is induced before administration of the antidiuretic hormone (1,2,4), much water can be reabsorbed first before significant sodium reabsorption becomes perceptible (as seen here in patients with CDI). However, several studies suggested that the natriuretic response to vasopressin is dependent on volume expansion induced by water retention (37) and/or results from an oxytocin-like effect induced by binding of vasopressin to oxytocin receptors (38).

With regard to differences in the agonists used, dDAVP was used here, not native vasopressin. Johnson *et al.* (39) showed that vasopressin and dDAVP infused in diuretic dogs increased urine osmolality to the same extent but that only vasopressin increased natriuresis and mean arterial pressure. Actually, with dDAVP infusion, V1a effects should totally disappear because the V2-mediated enhancement in water reabsorption likely reduces plasma osmolality and suppresses endogenous vasopressin secretion. Thus, this infusion likely resulted in a potent selective V2 stimulation in the absence of simultaneous V1a influence. This suggests that the natriuretic effects observed in other experiments result mostly from V1a effects, as suggested in recent rat studies (40).

To our knowledge, only one previous study evaluated the effects of dDAVP on renal function and solute excretion in humans (41). In two opposite conditions (expansion or depletion of extracellular volume), dDAVP produced a significant reduction in urinary flow rate, creatinine clearance, and sodium excretion. Part of the fall in sodium excretion was not GFR dependent because the fractional excretion of sodium was also decreased, thus indicating a tubular effect. Thus, this study, although performed in very different conditions from ours (and departing largely from normal physiologic conditions), supports the concept that dDAVP is antinatriuretic as a result of its action on the renal tubule.

In vivo, endogenous secretion of vasopressin should induce both V1a and V2 receptor–mediated effects if the two types of receptors are equally sensitive to the hormone. However, V2 effects seem to be more intensely activated than V1a effects when vasopressin varies within a physiologic range (19). Several studies showed that, in healthy humans, the basal sodium excretion or the ability to excrete a salt load is lower when urine flow rate is kept within a relatively low physiologic range than when it is artificially increased by abundant fluid intake (15,17,42,43). These observations suggest that endogenous vasopressin may actually also be antinatriuretic in humans. Moreover, an imbalance in the relative abundance, sensitivity, affinity, and/or signal transduction of V2 *versus* V1a receptors in some disease states might alter the normal balance between V2 and V1a influences. Noteworthy, studies in rats have shown that V1a but not V2 receptors are downregulated (44,45) in situations in which vasopressin levels are increased, such as in diabetes (46).

This study explored only the acute effects of V2 agonism and does not allow extrapolation to chronic situations, but a few experimental studies suggest that chronic V2 receptor–induced excessive sodium reabsorption could lead to sodium retention and to a compensatory rise in BP. Sustained infusion of dDAVP in rats for 1 to 2 wk resulted in a significant elevation of BP (by approximately 10 mmHg) (47,48) and in a worsening of DOCA

salt–induced hypertension (48). Because the same chronic dDAVP infusion increased ENaC mRNA (13) and protein (14) abundance and ENaC activity in isolated CD *in vitro* (13), it seems legitimate to assume that an enhanced ENaC-dependent sodium reabsorption might play a role in the rise in BP. In humans, several studies revealed an association between higher BP and/or reduced nocturnal BP dipping and a low urine flow rate (49–52). Chronic studies are obviously needed to evaluate more directly the possible contribution of an excessive stimulation of V2 receptors in hypertension.

In conclusion, this study shows that vasopressin may have a significant antinatriuretic effect in humans that depends selectively on V2 receptors. Because of the known actions of vasopressin on the CD *in vitro*, it seems reasonable to assume that this antinatriuretic effect results from an increase in ENaC-mediated sodium reabsorption in the CD. Further studies are required to confirm this hypothesis and to evaluate the consequences of this antinatriuretic effect in the long term.

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References

1. Balment RJ, Brimble MJ, Forsling ML, Musabayane CT: Natriuretic response of the rat to plasma concentrations of arginine vasopressin within the physiological range. *J Physiol* 352: 517–526, 1984
2. Humphreys MH, Friedler RM, Earley LE: Natriuresis produced by vasopressin or hemorrhage during water diuresis in the dog. *Am J Physiol* 219: 658–665, 1970
3. Park RG, Congiu M, Denton DA, McKinley MJ: Natriuresis induced by arginine vasopressin infusion in sheep. *Am J Physiol* 249: F799–F805, 1985
4. Forsling ML, Judah JM, Windle RJ: The effect of vasopressin and oxytocin on glomerular filtration rate in the conscious rat: Contribution to the natriuretic response. *J Endocrinol* 141: 59–67, 1994
5. Tomita K, Pisano JJ, Knepper MA: Control of sodium and potassium transport in the cortical collecting duct of the rat. *J Clin Invest* 76: 132–136, 1985
6. Reif MC, Troutman SL, Schafer JA: Sodium transport by rat cortical collecting tubule. Effects of vasopressin and desoxycorticosterone. *J Clin Invest* 77: 1291–1298, 1986
7. Macknight AD, DiBona DR, Leaf A: Sodium transport across toad urinary bladder: A model "tight" epithelium. *Physiol Rev* 60: 615–715, 1980
8. Verrey F: Antidiuretic hormone action in A6 cells: Effect on apical Cl and Na conductances and synergism with aldosterone for NaCl reabsorption. *J Membr Biol* 138: 65–76, 1994
9. Djelidi S, Fay M, Cluzeaud F, Escoubet B, Eugene E, Capurro C, Bonvalet JP, Farman N, Blot-Chabaud M: Transcriptional regulation of sodium transport by vasopressin in renal cells. *J Biol Chem* 272: 32919–32924, 1997
10. Capurro C, Rivarola V, Kierbel A, Escoubet B, Farman N, Blot-Chabaud M, Parisi M: Vasopressin regulates water flow in a rat cortical collecting duct cell line not containing known aquaporins. *J Membr Biol* 179: 63–70, 2001
11. Schafer JA: Abnormal regulation of ENaC: Syndromes of salt retention and salt wasting by the collecting duct. *Am J Physiol Renal Physiol* 283: F221–F235, 2002
12. Richardson DW, Robinson AG: Desmopressin. *Ann Intern Med* 103:228–239, 1985
13. Nicco C, Wittner M, DiStefano A, Jounier S, Bankir L, Bouby N: Chronic exposure to vasopressin upregulates ENaC and sodium transport in the rat renal collecting duct and lung. *Hypertension* 38: 1143–1149, 2001
14. Ecelbarger CA, Kim GH, Terris J, Masilamani S, Mitchell C, Reyes I, Verbalis JG, Knepper MA: Vasopressin-mediated regulation of epithelial sodium channel abundance in rat kidney. *Am J Physiol Renal Physiol* 279: F46–F53, 2000
15. Choukroun G, Schmitt F, Martinez F, Drüeke TB, Bankir L: Low urine flow reduces the capacity to excrete a sodium load in humans. *Am J Physiol* 273: R1726–R1733, 1997
16. Lieberthal W, Vasilevsky ML, Valari R, Levinsky N: Interactions between ADH and prostaglandins in isolated erythrocyte-perfused rat kidney. *Am J Physiol* 252: F331–F337, 1987
17. Bankir L, Pouzet B, Choukroun G, Bouby N, Schmitt F, Mallie JP: [To concentrate the urine or to excrete sodium: Two sometimes contradictory requirements.] *Néphrologie* 19: 203–209, 1998
18. Hawk CT, Li L, Schafer JA: AVP and aldosterone at physiological concentrations have synergistic effects on Na+ transport in rat CCD. *Kidney Int* 50[Suppl 57]: S35–S41, 1996
19. Bankir L: Antidiuretic action of vasopressin: Quantitative aspects and interaction between V1a and V2 receptor-mediated effects. *Cardiovasc Res* 51: 372–390, 2001
20. Bichet DG, Razi M, Lonergan M, Arthus MF, Papukna V, Kortas C, Barjon JN: Hemodynamic and coagulation responses to 1-desamino[8-D-arginine] vasopressin in patients with congenital nephrogenic diabetes insipidus. *N Engl J Med* 318: 881–887, 1988
21. Bichet DG, Razi M, Arthus MF, Lonergan M, Tittley P, Smiley RK, Rock G, Hirsch DJ: Epinephrine and dDAVP administration in patients with congenital nephrogenic diabetes insipidus. Evidence for a pre-cyclic AMP V2 receptor defective mechanism. *Kidney Int* 36: 859–866, 1989
22. Bichet DG, Arthus MF, Lonergan M: The hemodynamic and coagulant effects of dDAVP are specific extrarenal V2-receptor responses. In: *Desmopressin in Bleeding Disorders*, edited by Mariani G, New York, Plenum Press, 1993, pp 89–99
23. Bardoux P, Bichet DG, Martin H, Gallois Y, Marre M, Arthus MF, Lonergan M, Ruel N, Bouby N, Bankir L: Vasopressin increases urinary albumin excretion in rats and humans: Involvement of V2 receptors and the renin-angiotensin system. *Nephrol Dial Transplant* 18: 497–506, 2003
24. Bankir L, Trinh-Trang-Tan MM: Urea and the kidney. In: *The Kidney*, 6th Ed., edited by Brenner BM, Philadelphia, W.B. Saunders, 2000, pp 637–679

25. Field MJ, Giebisch GJ: Hormonal control of renal potassium excretion. *Kidney Int* 27: 379–387, 1985
26. Bouby N, Ahloulay M, Nsegbe E, Déchaux M, Schmitt F, Bankir L: Vasopressin increases GFR in conscious rats through its antidiuretic action. *J Am Soc Nephrol* 7: 842–851, 1996
27. Pozet N, Labeeuw M, Kaffa I, Hadj Aissa A, Cochat P, Zech P, Traeger J: [Creatinine clearance with low urinary output.] *Néphrologie* 6: 78, 1985
28. Reif MC, Troutman SL, Schafer JA: Sustained response to vasopressin in isolated rat cortical collecting tubule. *Kidney Int* 26: 725–732, 1984
29. Kaufmann JE, Oksche A, Wollheim CB, Gunther G, Rosenthal W, Vischer UM: Vasopressin-induced von Willebrand factor secretion from endothelial cells involves V2 receptors and cAMP. *J Clin Invest* 106: 107–116, 2000
30. VanLieburg AF, Knoers NV, Monnens LA, Smits P: Effects of arginine vasopressin and 1-desamino-8-D arginine vasopressin on forearm vasculature of healthy subjects and patients with a V2 receptor defect. *J Hypertens* 13: 1695–1700, 1995
31. Medina P, Segarra G, Vila JM, Chuan P, Domenech C, Lluch S: V2-receptor-mediated relaxation of human renal arteries in response to desmopressin. *Am J Hypertens* 12: 188–193, 1999
32. Bankir L, de Rouffignac C: Urinary concentrating ability: Insights from comparative anatomy. *Am J Physiol* 249: R643–R666, 1985
33. Chabardes D, Gagnan-Brunette M, Imbert-Teboul M, Gontcharevskaia O, Montegut M, Clique A, Morel F: Adenylate cyclase responsiveness to hormones in various portions of the human nephron. *J Clin Invest* 65: 439–448, 1980
34. Ruggles BT, Murayama N, Werness JL, Gapstur SM, Bentley MD, Dousa TP: The vasopressin-sensitive adenylate cyclase in collecting tubules and in thick ascending limb of Henle's loop of human and canine kidney. *J Clin Endocrinol Metab* 60: 914–921, 1985
35. Schafer JA: The rat cortical collecting duct as an isosmotic volume reabsorber. In: *Isotonic Transport in Leaky Epithelia*, edited by Ussing HH, Fischbarg J, Sten-Knudsen O, Larsen FH, Willumsen NJ, Copenhagen, Alfred Benzon Symposium, 1993, pp 339–350
36. Valtin H: Sequestration of urea and nonurea solutes in renal tissues of rats with hereditary hypothalamic diabetes insipidus: Effect of vasopressin and dehydration on the countercurrent mechanism. *J Clin Invest* 45: 337–345, 1966
37. Walter SJ, Tennakoon V, McClune JA, Shirley DG: Role of volume status in vasopressin-induced natriuresis: Studies in Brattleboro rats. *J Endocrinol* 151: 49–54, 1996
38. Brimble MJ, Balment RJ, Smith CP, Windle RJ, Forsling ML: Influence of oxytocin on sodium excretion in the anaesthetized Brattleboro rat. *J Endocrinol* 129: 49–54, 1991
39. Johnson MD, Kinter LB, Beeuwkes R 3rd: Effects of AVP and DDAVP on plasma renin activity and electrolyte excretion in conscious dogs. *Am J Physiol* 236: F66–F70, 1979
40. Bankir L, Bardoux P, Fernandes S, Bouby N: Dissociation between natriuretic and antinatriuretic effects of vasopressin: respective roles of V1a and V2 receptors, as studied in conscious normally hydrated rats [Abstract]. *J Am Soc Nephrol* 16: 87A, 2004
41. Agnoli GC, Borgatti R, Cacciari M, Lenzi P, Marinelli M, Stipo L: Low-dose desmopressin infusion: Renal action in healthy women in moderate salt retention and depletion, and interactions with prostanoids. *Prostaglandins Leukot Essent Fatty Acids* 67: 263–273, 2002
42. Andersen LJ, Andersen JL, Schütten HJ, Warberg J, Bie P: Antidiuretic effect of subnormal levels of arginine vasopressin in normal humans. *Am J Physiol* 259: R53–R60, 1990
43. Anastasio P, Cirillo M, Spitali L, Frangiosa A, Pollastro RM, De Santo NG: Level of hydration and renal function in healthy humans. *Kidney Int* 60: 748–756, 2001
44. Trinder D, Phillips PA, Stephenson JM, Risvanis J, Aminian A, Adam W, Cooper M, Johnston CI: Vasopressin V1 and V2 receptors in diabetes mellitus. *Am J Physiol* 266: E217–E223, 1994
45. Phillips PA, Risvanis J, Hutchins AM, Burrell LM, MacGregor D, Gundlach AL, Johnston CI: Down-regulation of vasopressin V1a receptor mRNA in diabetes mellitus in the rat. *Clin Sci (Lond)* 88: 671–674, 1995
46. Bankir L, Bardoux P, Ahloulay M: Vasopressin and diabetes mellitus. *Nephron* 87: 8–18, 2001
47. Montani JP, Wang JL, Tempini A, Van Vliet BN: Chronic infusion of a V2-vasopressin agonist results in sustained hypertension in rats [Abstract]. *FASEB J* 14: A675, 2000
48. Fernandes S, Bruneval P, Hagege A, Heudes D, Ghostine S, Bouby N: Chronic V2 vasopressin receptor stimulation increases basal blood pressure and exacerbates deoxycorticosterone acetate-salt hypertension. *Endocrinology* 143: 2759–2766, 2002
49. Berecek KH, Brody MJ: Vasopressin and deoxycorticosterone hypertension in Brattleboro rats. *Ann N Y Acad Sci* 394: 319–329, 1982
50. Bankir L, Bardoux P, Mayaudon H, Dupuy O, Bauduceau B: Too low urinary flow rate during the day: New factor possibly involved in hypertension and in the lack of nocturnal dipping [Abstract]. *J Hypertens* 20: A7, 2002
51. Bankir L, Sellin F, Chiolero A, Burnier M: A reduced nocturnal dipping in blood pressure in patients with moderate essential hypertension is associated with a disturbed diurnal/nocturnal pattern of water and sodium excretion [Abstract]. *J Am Soc Nephrol* 14: 20A–21A, 2003
52. Bankir L, Bouby N, Sellin F, Fineberg N, Weinberger M: Are racial differences in sodium and water handling at night related to differences in the susceptibility to hypertension [Abstract]? *J Hypertens* 22[Suppl 2]: S216–S217, 2004