Vasopressin increases urinary albumin excretion in rats and humans: involvement of V2 receptors and the renin–angiotensin system

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Abstract

Background. An increase in urinary albumin excretion (UAE) represents an early predictor of glomerular damage in diabetes mellitus (DM) and a risk factor for cardiovascular complications in hypertension. Vasopressin is elevated in DM and in some forms of hypertension. Previous studies in rats suggested that this hormone could play a role in the albuminuria observed in chronic renal failure or diabetic nephropathy, but no information is available concerning the mechanism of these effects and the possible influence of vasopressin on UAE in the healthy kidney. The present study was thus designed to evaluate whether vasopressin influences UAE in normal rats and humans, whether this effect is V2-receptor-dependent, and whether it is mediated by the renin–angiotensin system.

Methods. UAE was measured in normal Wistar rats and healthy humans, or in subjects with various forms of diabetes insipidus (DI), before and after acute or chronic infusion of the vasopressin V2 receptor agonist dDAVP1. Chronic dDAVP administration was also performed in normal Wistar rats previously submitted to either chronic angiotensin-converting enzyme inhibition (ACEI) or chronic blockade of AT1 receptors (ARB).

Results. In rats, acute or chronic dDAVP infusion increased UAE significantly and reversibly (4-fold and 6-fold, respectively). In healthy subjects, acute infusion of dDAVP tripled UAE (P < 0.01) but did not change creatinine and β2-microglobulin excretion, thus suggesting that the rise in UAE was due to an increased glomerular leakage of albumin. dDAVP also increased UAE in patients with central DI and in patients with hereditary nephrogenic DI bearing AQP2 mutations. However, UAE was not increased in patients with hereditary nephrogenic DI bearing mutations of the V2 receptor. In rats, ACEI and ARB blunted the dDAVP-induced rise in UAE by 70% (P < 0.05) and 50% (NS), respectively.

Conclusions. The present studies reveal for the first time that vasopressin induces a marked increase in UAE in healthy rats and humans. This albuminuric effect seems to result from increased glomerular leakage, requires functional vasopressin V2 receptors, and is, at least in part, mediated by the renin–angiotensin system. These results bring additional support for an involvement of vasopressin in the albuminuria observed in pathological states such as diabetes mellitus or hypertension.

Keywords: ACE inhibition; angiotensin II receptor blockade; blood pressure; diabetes insipidus; diabetes mellitus; microalbuminuria

Introduction

An increase in urinary albumin excretion (UAE) is widely recognized as an early predictor of glomerular damage, especially in diabetes mellitus (DM). In hypertension, it also identifies patients at greater risk of cardiovascular complications. The contribution of the renin–angiotensin system in the mechanism responsible for glomerular leakage of albumin is well established and indirectly confirmed by the beneficial effects of angiotensin-converting enzyme inhibitors (ACEI) or angiotensin-II-receptor blockers (ARB) in patients with hypertension and/or various nephropathies. In rats, another hormone, vasopressin (VP) or its V2 receptor agonist dDAVP1, have also been shown, to contribute to hypertension, single nephron hyperfiltration, urinary protein excretion, and to progression of renal failure [1–3]. VP or dDAVP also increases glomerular filtration rate (GFR) [4] and the glomerular ultrafiltration coefficient Kf in normal rats, and
induces a significant hypertrophy of the kidney similar to that seen in response to high protein intake [5]. However, to our knowledge, no study has evaluated the possible effects of VP or dDAVP on UAE in the absence of renal disease, whether in rats or in humans. 

Diabetic nephropathy is a frequent complication of DM. Early changes in renal function during DM include hyperfiltration, renal enlargement and microalbuminuria. A marked increase in VP secretion and VP plasma level is well documented in DM [6]. In a previous study, we showed that VP participates in the early manifestations of diabetic nephropathy [7]. Experimental type I DM was induced in Brattleboro rats, unable to secrete VP because of a deletion in the VP gene. Glomerular hyperfiltration, renal hypertrophy, and the progressive rise in albuminuria were absent or much less intense in these rats than in their VP-replete Long–Evans controls [7]. 

Taken together, these findings strongly suggest that VP plays a role in the induction of albuminuria preceding and accompanying progressive renal damage due either to severe reduction in renal mass or to diabetic nephropathy. The mechanism by which VP can induce this effect is, however, unclear. Whether the elevation in UAE is due to a glomerular leakage of albumin or to a defect in its tubular reabsorption is not known. V2 receptors are most probably involved (i) because renal V1a but not V2 receptors have been shown to be downregulated in DM and (ii) because dDAVP, which induces hyperfiltration in normal rats [4], is a V2 receptor agonist with low affinity for V1a receptors [8]. However, V2 receptors or their mRNA have not been observed in glomeruli or proximal tubules [9]. This suggests that VP effects on albuminuria and hyperfiltration are indirect, as already discussed for VP-induced hyperfiltration [4,10]. They could result from associated changes in blood pressure because proteinuria and blood pressure were observed to vary in parallel when the VP–water intake axis was altered in rats with chronic renal failure [1,3]. A contribution of the renin–angiotensin system, activated in response to changes in the tubulo-glomerular feedback was also suspected to contribute to the haemodynamic effects of VP [4,10]. 

The present study was designed to evaluate whether VP has an influence on albumin excretion through a V2-receptor-dependent pathway in rats and humans with normal kidney function, and whether the renin–angiotensin system and/or associated changes in blood pressure mediate these effects. First, changes in albumin excretion in response to acute or chronic administration of dDAVP were evaluated in normal rats. Second, albumin excretion was measured after short-term infusion of dDAVP in healthy humans and in subjects with hereditary diabetes insipidus due to different aetiologies. Third, the possible contribution of the renin–angiotensin system to dDAVP-induced albuminuria was evaluated in rats by studying the effect of dDAVP after chronic blockade of this system by converting-enzyme inhibition or by selective antagonism of angiotensin II AT1 receptors. 

Subjects and methods

In all rat and human studies presented below, the VP peptidic analogue dDAVP (1-desamino 8-D-arginine vasopressin, desmopressin) (Ferring Pharmaceuticals, AB, Malmö, Sweden) was used rather than native VP, in order to study selectively the effects mediated by V2 receptors and to avoid those mediated by potentially vasopressor V1a receptors. 

Studies in rats

Adult male Wistar rats [body weight (BW) 230–240 g], purchased from Iffa Credo (L’Arbresle, France), were housed individually in metabolic cages during the whole experiments with free access to tap water and powdered food (A03, UAR, Epinay-Orge, France) except when otherwise mentioned. They were allowed at least 4 days of adaptation before starting the experiments.

Experimental protocols

Acute infusion of dDAVP (Experiment A). Eight rats were studied during 3 successive days. On the first 2 days, at 8:00 a.m., rats received an intraperitoneal (i.p.) injection of either 500 μl isotonic saline (basal day) or 500 ng dDAVP in 500 μl isotonic saline (experimental day). No injection was given on the third, recovery, day. On each day, urine was collected from 8:00 a.m. to 2:00 p.m. (6 h following the injection) and from 2:00 p.m. to 8:00 a.m. of the following day (18 subsequent hours). Urine flow rate (V), urine osmolality (Um) and urinary albumin concentration (Uab) were measured (see below).

Chronic infusion of dDAVP (Experiment B). In experiment B, seven rats were studied during 3 successive weeks (basal, experimental, and recovery weeks). During the experimental week, rats were infused with dDAVP, 8.33 ng/h (200 ng/day), delivered at a rate of 1 μl/h through osmotic minipumps (model 2001, Alza Corporation, Palo Alto, USA) implanted i.p. under brief anaesthesia (ketamine, 8.33 mg/kg/day, xylazine, 0.5 mg/kg/day). In experiment B, seven rats were studied during 3 successive days. On the first 2 days, at 8:00 a.m., rats received an intraperitoneal (i.p.) injection of either 500 μl isotonic saline (basal day) or 500 ng dDAVP in 500 μl isotonic saline (experimental day). No injection was given on the third, recovery, day. On each day, urine was collected from 8:00 a.m. to 2:00 p.m. (6 h following the injection) and from 2:00 p.m. to 8:00 a.m. of the following day (18 subsequent hours). Urine flow rate (V), urine osmolality (Um) and urinary albumin concentration (Uab) were measured (see below).
of food per day (25 g), corresponding roughly to the previously measured average food intake of rats of this BW. After 4 days of adaptation, renal function was studied for 2 consecutive days and rats were divided into two equal groups of equivalent BW, urinary concentrating activity and UAE. One of the groups was then given Coversen (tert-butylamine salt of perindopril, Servier, Orléans, France) at a dose of 1 mg/100 g BW per day, mixed in the powdered food. Ten days after initiation of the treatment, the same 3-week protocol as that described for Experiment B was performed. In addition, systolic blood pressure (SBP) was measured by the tail-cuff method (BP recorder 8005, Apleex, Massy, France) 3 days per week, starting during the first week of ACEI treatment. Rats were placed for 20 min under a specially adapted, thermostatic heating element before measurements. Results of the last 2 days of each week were averaged for each rat.

In experiment D, the possible influence of angiotensin II was blocked by chronic administration of the AT1 receptor antagonist losartan (MSD, Wilmington, USA) added to the drinking water so as to provide a daily dose of 3 mg/100 g BW (the concentration of losartan in the drinking water was increased during the dDAVP week in order to compensate for the reduction in fluid intake induced by this hormone). The losartan treatment was started 8 days before the beginning of urine collection. The same 3-week protocol as that described for experiments B and C was then performed (blood pressure was not measured in experiment D).

Studies in human subjects

Subjects. In previous studies at the Hôpital du Sacré-Cœur (Montreal), healthy subjects and patients with hereditary diabetes insipidus (DI) of different aetiologies had been submitted to a dDAVP infusion to evaluate the influence of this drug on coagulation factors and plasma cyclic adenosine monophosphate (cAMP). The following protocol had been used (as reported in [11]). dDAVP, 0.3 μg/kg BW, was infused i.v. 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. Mean arterial blood pressure was measured every 30 min. Blood samples were taken every 10 min for the first 30 min after initiation of dDAVP infusion and every 30 min thereafter, for measurement of plasma renin activity. Urinary flow rate and osmolality, and urinary creatinine concentration were measured, and representative aliquots of urine were frozen at −70°C.

In the present study, we reconsidered six healthy subjects and 15 DI patients for whom frozen urine samples were still available. In urine of healthy subjects (3 male, 3 female, 31 ± 4 years, 67 ± 6 kg), we measured urinary albumin and β2-microglobulin concentrations. We also report plasma renin activity (PRA), urinary flow rate and osmolality, and creatinine excretion (not reported in the initial paper), and mean arterial blood pressure (MAP) [11]. Albumin concentration was also evaluated in urine of four of these healthy subjects collected with the same timing during a sham test involving infusion of isotonic saline instead of dDAVP.

Among the patients with DI, two had central DI (CDI) and 13 had nephrogenic DI (NDI) due to mutations of either the vasopressin V2 receptor gene (NDI-VPR2, n = 10) or the aquaporin 2 gene (NDI-AQP2, n = 3). The mutations involved are shown in Table 2. CDI patients stopped their dDAVP for 24 h and NDI patients stopped their hydrochlorothiazide medication for 3 days. Urinary albumin concentration was measured in urine collected from these DI patients before and after dDAVP infusion, with the same timing as in healthy subjects.

Although urine samples had been stored for 8–9 years, albumin and β2-microglobulin had not undergone significant degradation because values found here in healthy subjects fell within the range of values usually reported for fresh urine. In any case, a poor conservation of the samples could only have induced an underestimation of albumin excretion and could not explain the increases seen after dDAVP infusion or the high values observed in some of the DI subjects (see Results).

Biochemical analyses and statistics. In rat studies, urine osmolality was measured with a freezing-point microosmometer (Roebeling, Berlin, Germany), and urea concentration in plasma and urine with a commercial kit (Urea kit, Biomerieux, Lyon, France). Albumin concentration in rat urine was measured by radial immunodiffusion using an antisera against rat albumin raised in rabbit (Nordic Immunology, Tilburg, The Netherlands). Albumin and β2-microglobulin concentrations in human urine were measured by nephelometry with appropriate specific antibodies (Dade Behring, Marburg, Germany).

Results are expressed as means ± SEM. All urine data in rat studies of experiments B, C and D are means of the two successive 24-h urine collections for each rat. Data were analysed by ANOVA with repeated measures followed by Fisher post-hoc test. In healthy humans, the influence of dDAVP was evaluated by ANOVA with repeated measures followed by Dunnett post-hoc test, comparing values observed at each time after the beginning of dDAVP infusion to those obtained during the basal period (mean of the first two 30-min urine collections). For patients with DI, only descriptive statistics are provided because the number of subjects was too small in two of the groups to allow valid statistical comparisons.

Results

Influence of dDAVP in normal rats

The effects of the acute infusion of dDAVP in normal rats (experiment A) are shown in Table 1. As expected, dDAVP induced a significant increase in US (±47%, P < 0.001) and decrease in V (−34%, P < 0.001) in the 6-h period following the injection. Simultaneously, UAE rose by 3.2-fold on the average (P < 0.01). In the next 18 h, albuminuria returned to values identical to those of the same 18 h period of the basal day, in parallel with US and V, and remained so for the next 6-h period of the following day (recovery). Notably, three of the eight rats did not increase their UAE at all and their antidiuretic response to dDAVP was weaker than that in the five other rats (+531 vs +954 mOsm/kg H2O) although they had a normal basal urine osmolality.

The chronic effects of dDAVP are shown in Table 1 (experiment B). Plasma sodium concentration fell by 3 mmol/l during the dDAVP week (from 143 ± 0 to 140 ± 1 mmol/l, P < 0.01) and returned to control
In the whole, the antidiuretic effects were of similar magnitude to those observed after acute dDAVP administration. The albuminuric effect, however, was more intense (5.8-fold increase, \( P - 0.01 \)).

Table 1. Influence of acute and chronic dDAVP administration on urinary concentrating activity and albumin excretion in rats

<table>
<thead>
<tr>
<th>Experiment A: acute study ( (n = 8) )</th>
<th>UAE (mg/6h)</th>
<th>( U_{\text{osm}} ) (mosmol/kg H(_2)O)</th>
<th>( V ) (ml/6h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal day</td>
<td>0.15 ± 0.02</td>
<td>1690 ± 150</td>
<td>3.24 ± 0.30</td>
</tr>
<tr>
<td>dDAVP day</td>
<td>0.48 ± 0.12**</td>
<td>2480 ± 160***</td>
<td>2.15 ± 0.25***</td>
</tr>
<tr>
<td>Recovery day</td>
<td>0.15 ± 0.01**</td>
<td>1730 ± 140***</td>
<td>3.49 ± 0.32***</td>
</tr>
<tr>
<td>dDAVP/basal</td>
<td>3.20</td>
<td>1.47</td>
<td>0.66</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Experiment B: chronic study ( (n = 7) )</th>
<th>UAE (mg/d)</th>
<th>( U_{\text{osm}} ) (mosmol/kg H(_2)O)</th>
<th>( V ) (ml/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal week</td>
<td>0.56 ± 0.05</td>
<td>1880 ± 180</td>
<td>15.8 ± 1.7</td>
</tr>
<tr>
<td>dDAVP week</td>
<td>3.26 ± 0.77**</td>
<td>2840 ± 140***</td>
<td>10.3 ± 0.5**</td>
</tr>
<tr>
<td>Recovery week</td>
<td>0.82 ± 0.06**</td>
<td>1550 ± 210***</td>
<td>20.6 ± 1.9***</td>
</tr>
<tr>
<td>dDAVP/basal</td>
<td>5.82</td>
<td>1.51</td>
<td>0.65</td>
</tr>
</tbody>
</table>

Values are means ± SEM of \( n \) rats per experiment. ANOVA with repeated measures and Fisher post-hoc test: **\( P < 0.01 \), ***\( P < 0.001 \) vs preceding day (in experiment A) or week (in experiment B).

Influence of dDAVP in humans

The influence of acute dDAVP administration in healthy subjects is shown in Figure 1. \( V \) fell markedly in the first half-hour and remained low for the remaining 2 h. \( U_{\text{osm}} \), which was already relatively high in the basal period (680 ± 110 mOsm/kg H\(_2\)O), rose only modestly (up to 940 ± 80 at 180 min), and thus, osmolar excretion fell to about half of the basal value. Albumin excretion increased significantly in all subjects during the first hour (\( P < 0.01 \)) but the intensity of this increase varied largely from one subject to the other (from 70 to 600%). During the second and third hours, UAE returned to values close to basal level. Creatinine and \( \beta_2 \)-microglobulin excretions fell slightly in the first half-hour, in parallel with the reduction in \( V \). In four subjects who received a
sham infusion on another occasion, no change in urine osmolality, urine flow rate, or albumin excretion was observed over 180 min (data not shown).

As described previously by Bichet et al. [11], dDAVP infusion induced an abrupt fall in MAP (associated with facial flushing), a rise in PRA (Figure 1) and heart rate (data not shown). These changes preceded the rise in UAE. Increases in plasma atrial natriuretic peptide (ANP), cAMP, and cyclic guanosine monophosphate (cGMP) concentrations also occurred (data not shown). However, they exhibited a lesser intensity (25–30% increase for ANP and cGMP, and 50% for cAMP), reached their maximum after only 60–120 min, and did not decline thereafter.

Figure 2 shows the results observed in patients with diverse forms of DI. The two patients with central DI exhibited the expected antidiuretic response to dDAVP infusion. A very intense rise in UAE was also observed, largely exceeding that seen in healthy subjects. From 90 to 150 min after the dDAVP infusion, UAE reached a plateau at a level 12- and 31-fold above values recorded in the basal period. In patients with NDI, no antidiuretic response was observed (as could be expected). However, there was a distinct rise in UAE in those with AQP2 mutations but no or only a very modest rise in nine of the 10 patients with mutations of the V2 receptor (Table 2). Note that four of the 13 patients with NDI exhibited unusually high basal UAE, ~5–10 times higher than that in healthy subjects and other DI patients (Figure 2 and Table 2).

**Influence of previous blockade of the renin–angiotensin system on dDAVP-induced albuminuria in rats**

To evaluate the possible contribution of the renin-angiotensin system in the effects of dDAVP on albumin excretion, dDAVP was infused chronically in rats previously submitted to chronic ACEI or blockade of AT1 receptors (Figures 3 and 4, respectively). Treatment with perindopril markedly lowered SBP in the basal week (~26 mmHg, P < 0.001). SBP was not measured in rats receiving losartan, but it was shown to fall by 30 mmHg in Wistar rats treated in the same way in a previous experiment performed in the same laboratory [12]. Thus, it may be assumed that SBP fell similarly in ACEI- and ARB-treated rats of the present experiment. No change in SBP occurred during the week of dDAVP infusion, but a modest fall was observed after discontinuation of this infusion (P < 0.01) (Figure 3).

ACEI did not influence either the basal urine-concentrating activity (U\text{osm} and \upsilon) or the antidiuretic response to dDAVP (Figure 3); osmolar excretion was similar in both groups and unaltered by chronic dDAVP infusion (data not shown). In contrast, ACEI largely blunted the rise in UAE compared to that observed in control rats (which was similar to that seen in experiment B). UAE rose by 0.75 ± 0.24 mg/day in ACEI-rats (~2.1 compared with basal) vs 2.35 ± 0.67 mg/d in control rats (~3.9 compared with basal) (P < 0.05). All values returned to control levels after discontinuation of dDAVP infusion (Figure 3).

**Fig. 2.** Effect of acute dDAVP administration on urine flow rate (\upsilon), urine osmolality (U\text{osm}), and urinary excretion of albumin (UAE) in patients with different forms of diabetes insipidus. Results are means (left and middle panels) or means ± SEM (right panel) of n subjects. Values shown at time 0 were obtained by averaging results from two 30-min basal periods in each patient. Four patients with NDI exhibited very high basal UAE and are shown separately from other patients (open symbols). As can be seen in the figure, they did not differ from other patients regarding the lack of antidiuretic response to dDAVP. Some SEM are not visible in the right panel because they are smaller than the symbols.
In experiment D, chronic blockade of AT1 receptors did not alter basal urinary concentration or the antidiuretic response to dDAVP (data not shown). U\textsubscript{osm} and V\textsubscript{d} during the 3 weeks in the two groups were very similar to those in experiment C (data not shown). UAE rose by 2.70 ± 0.77 mg\textsubscript{u} day during dDAVP infusion in the control group (35.8 compared with basal), and by only 1.24 ± 0.30 mg\textsubscript{u} day in the group receiving the ARB (33.0 compared with basal) (Figure 4). The difference in the dDAVP-induced rise in UAE between the two groups did not reach statistical significance. Thus, both ACEI and ARB reduced the dDAVP-mediated albuminuria but this effect was weaker with the latter.

Plasma urea increased significantly during dDAVP infusion in all groups (Table 3). This increase was due to a more intense urea reabsorption known to occur in the collecting duct at low urine flow rates and to the vasopressin-dependent diffusion of urea from the terminal collecting ducts into the medullary interstitium. Part of this urea fails to be recycled within the kidney and thus returns to the general circulation [10].
Discussion

The present study shows for the first time that the V₂ actions of VP induce an increase in urinary albumin excretion in both rats and humans. This albuminuric effect is most probably indirect and probably involves the intrarenal renin-angiotensin system.

Albumin excretion is usually kept to a very low level, due to the relatively low permeability of the glomerular filtration barrier to albumin, and to efficient reabsorptive mechanisms in the proximal tubule. An increase in UAE is interpreted as a sign of early renal damage in several diseases including DM and hypertension. The present results suggest that VP could contribute to albuminuria in these pathological situations involving a rise in its plasma level.

The fact that β₂-microglobulin excretion did not rise in parallel with that of albumin in healthy humans suggests that the dDAVP-induced rise in UAE originated from an increased leakage of albumin through the glomerular filtration barrier rather than from a reduction in its tubular reabsorption. The rise in UAE was transient. This may be due, at least in part, to the fact that dDAVP concentration in plasma fell with time after cessation of the 20-min infusion. In contrast, the antidiuretic effects lasted for several hours, most probably because the kidney’s architecture slows the dissipation of the gradient of solutes accumulated in the renal medulla. These different time courses suggest that dDAVP-induced albuminuria does not result from the physicochemical changes occurring in the kidney in relation to the process of urine concentration. The dDAVP-induced rise in UAE in normal rats was greater after a prolonged treatment than after a single injection. This suggests that dDAVP-dependent albuminuria may progressively worsen with prolonged exposure to the hormone.

In healthy humans, a marked fall in MAP and rise in PRA was observed in the first 30 min after dDAVP infusion, as already reported by Bichet and colleagues [11,13]. The fall in blood pressure may be due to extrarenal V₂ receptor-mediated vasodilatation [14] and may induce a rise in renin release [11,13,15] which contributes to the prompt restoration of blood pressure. The rise in PRA observed here was also short-lived but preceded the rise in albumin excretion. However, it may be assumed that the change in UAE was concomitant with that in PRA but was observed later due to the dead space in the urinary tract. The change in glomerular permeability to albumin was thus most probably synchronous with the presence of exogenous dDAVP in the blood and to the increased activity of the renin-angiotensin system. The precise mechanism by which these hormonal systems influence glomerular permselectivity cannot be inferred from the present study.

Table 3. Influence of chronic dDAVP administration on plasma urea concentration (mmol/l) in control rats, ACEI-treated rats and ARB-treated rats

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Week</th>
<th>Control (n = 8)</th>
<th>ACEI (n = 8)</th>
<th>ACEI/control</th>
<th>ARB (n = 8)</th>
<th>ARB/control</th>
</tr>
</thead>
<tbody>
<tr>
<td>E: ACEI (perindopril)</td>
<td>Basal week</td>
<td>6.0 ± 0.2</td>
<td>6.8 ± 0.3</td>
<td>1.13</td>
<td>8.5 ± 0.6</td>
<td>1.08</td>
</tr>
<tr>
<td></td>
<td>dDAVP week</td>
<td>10.2 ± 0.4***</td>
<td>15.6 ± 0.8***,###</td>
<td>1.53</td>
<td>11.8 ± 0.6***</td>
<td>1.13</td>
</tr>
<tr>
<td></td>
<td>Recovery week</td>
<td>7.5 ± 0.2***</td>
<td>7.8 ± 0.4***</td>
<td>1.03</td>
<td>9.0 ± 0.3***</td>
<td>1.05</td>
</tr>
<tr>
<td>F: ARB (losartan)</td>
<td>Basal week</td>
<td>7.9 ± 0.5</td>
<td>8.5 ± 0.6</td>
<td>1.08</td>
<td>8.6 ± 0.3***</td>
<td>1.05</td>
</tr>
<tr>
<td></td>
<td>dDAVP week</td>
<td>10.4 ± 0.4***</td>
<td>11.8 ± 0.6***</td>
<td>1.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Recovery week</td>
<td>8.6 ± 0.3***</td>
<td>9.0 ± 0.3***</td>
<td>1.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SEM of n rats per group. ANOVA with repeated measures and Fisher post-hoc test: ***P < 0.001 vs preceding week in the same group, ###P < 0.001 vs control rats during the same week.
dDAVP infusion markedly increased UAE in all patients with DI who had functional V₂ receptors, whether they were able to concentrate urine in response to vasopressin (CDI) or not (NDI-AQP2). In contrast, dDAVP failed to increase (or increased only very modestly) UAE in patients with mutated, non-functional V₂ receptors. These results thus clearly establish that functional V₂ receptors are required for the manifestation of dDAVP-induced albuminuria. Interestingly, these patients also exhibited no change in blood pressure, PRA, and heart rate in response to dDAVP, as reported earlier [11,13].

The reason why basal UAE was markedly higher in four of the 13 NDI patients is unknown. These patients had normal creatinine clearance and no sign of renal insufficiency, except patient no. 7 (BER Mi) who had a creatinine clearance of 40 ml/min. It may be worth mentioning that Brattleboro rats with hereditary CDI also tend to have higher basal UAE and much greater individual variability in UAE than Long–Evans controls with normal vasopressin secretion [7]. Of note also, the dDAVP-induced rise in UAE tended to be more intense and was more prolonged in DI patients (with functional V₂ receptors) than in healthy subjects (compare Figures 2 and 3). The possibility that sustained production of dilute urine may favour glomerular and/or tubular leakage of albumin needs to be evaluated in further studies.

Possible mechanism responsible for the albuminuric effect of dDAVP

The influence of dDAVP on glomerular leakage is probably indirect because no V₂ receptors or V₂-receptor mRNA have been noted in the glomerulus [9]. More probably this influence depends on a sequence of events initiated by the action of the drug on renal tubular V₂ receptors. However, an antidiuretic response is not a prerequisite for this albuminuric effect, because it was also observed in NDI-AQP2 patients who were unable to concentrate urine after dDAVP administration. Vasodilatory effects mediated by extra-renal V₂ receptors [14] may also be involved. The present experiments do not provide any information on these intermediate steps. However, several observations suggest that an activation of the renin–angiotensin system could participate in this mechanism. Angiotensin II is known to increase the glomerular filtration of albumin or proteins in vivo or in the isolated kidney and to enhance glomerular albumin permeability in isolated glomeruli in vitro [16]. A marked rise in PRA was observed in healthy subjects just before the rise in UAE in the present study, and patients with NDI-VPR2, who failed to exhibit a rise in UAE, also showed no change in PRA.

Activation of the intrarenal renin–angiotensin system may induce glomerular hypertension and thus favour albumin leakage, even in the absence of systemic hypertension [17]. In a previous study on the influence of VP or dDAVP on GFR, an inhibition of the activity of the tubulo-glomerular feedback (TGF) was suspected. This inhibition should result from a decrease in the sodium concentration of the tubular fluid at the macula densa, due to the more intense vasopressin-dependent intrarenal urea recycling that brings more urea in the lumen of loops of Henle [6,10].

The involvement of the intrarenal renin–angiotensin system in the TGF-dependent regulation of GFR is well established. The present study suggests that V₂-mediated actions of VP, in addition to increasing GFR and kidney weight [4,5], also enhance the glomerular leakage of albumin through a mechanism involving the renin–angiotensin system. Accordingly, VP probably activates the previously described vicious circle that leads from hyperfiltration and/or glomerular hypertension to progressive deterioration of renal function [18].

The results obtained here in rats submitted to either ACEI or ARB confirm the involvement of the renin–angiotensin system in the V₂ receptor-dependent rise in albuminuria. When angiotensin II actions were prevented by either approach, this rise was largely blunted. The slightly lower inhibition with losartan than with perindopril could be due either to small inter-experiment differences or to differing pharmacokinetics of the two drugs. Alternatively, it could suggest the involvement of additional factors suppressed by the latter but not by the former. Bradykinin, the half-life and thus the functional effects of which are potentiated by ACEI, may thus have played a role, through a mechanism that remains to be determined. Similarly, it is not possible to exclude a direct influence of angiotensin-converting enzyme, which is expressed in the nephron (especially along the brush border in the proximal tubule). However, even during efficient blockade of the renin–angiotensin system, a residual rise in albumin excretion after dDAVP exposure was still observed. This suggests either that the renin–angiotensin system was not fully blocked by the treatments (this is unlikely in view of the marked fall in blood pressure they induced), or that an additional mechanism, independent of the renin–angiotensin system, participated in the effect of dDAVP.

VP has been shown to stimulate the secretion of ANP, a hormone that could also induce a rise in UAE [19]. However, it seems unlikely that the dDAVP-induced rise in UAE could be mediated by ANP in the present study because of the very different time course and intensity of the rises observed in ANP and in UAE in healthy subjects.

Expected consequences in diverse pathological situations

The results of this study suggest that VP could contribute to albuminuria in pathological situations in which VP secretion is enhanced. This is the case in DM. Patients with uncontrolled type 1 or type 2 DM exhibit elevated plasma VP concentration. VP is also elevated in rats with either streptozotocin-induced DM or with a genetic form of DM (BB rats) [6]. Moreover,
basal levels of VP in type 2 diabetic patients showing albuminuria were reported to be significantly higher than in diabetics without complications [20]. Experimental data in rats strongly support the notion that endogenous VP contributes to the albuminuria of streptozotocin-induced DM. First, the usual rise in UAE was almost completely blunted when DM was induced in VP-deficient Brattleboro rats [7]. Second, chronic administration of a selective, non-peptide V₂ receptor antagonist (SR 121463, Sanofi-Recherche) to Wistar rats with DM totally prevented the rise in UAE seen in untreated rats [21]. In the present experiments, only V₂ effects of vasopressin were considered because the V₂ agonist, dDAVP, had been shown to induce hyperfiltration in normal rats [4] and it was thus interesting to see whether the same VP agonist would also increase albuminuria. Moreover, studies in rats and humans have shown that V₁a receptor are down-regulated in diabetes mellitus [6]. However, a recent study showed that 3-week treatment with a vasopressin V₁a antagonist induced a modest decrease in albuminuria in diabetic patients, thus suggesting that the V₁a effects of the hormone, if still significant, could also increase albumin excretion [20].

Because VP effects on albuminuria appear to be mediated by the renin–angiotensin system, it is conceivable that the protection against progression of microalbuminuria and diabetic nephropathy afforded by ACEI or ARBs could be due, at least in part, to the interruption of a V₂-receptor-dependent renal action [6]. Another situation in which VP is chronically elevated is the syndrome of inappropriate secretion of antidiuretic hormone. To our knowledge, no study has evaluated UAE in such patients. Similarly, no information is available regarding UAE in patients with central DI who are chronically treated with dDAVP.

Besides DM, albuminuria also represents a risk factor for cardiovascular morbidity and mortality in hypertension. Now, an elevated VP plasma level has been documented in some groups of hypertensive patients [22,23]. High VP secretion is also observed in Sabra rats, a model of salt-sensitive hypertension [24]. Thus, it may be assumed that VP also contributes to enhanced albumin excretion associated with high blood pressure. Interestingly, smoking, a proven risk factor in hypertension and diabetic nephropathy, is also a potent stimulus for VP secretion.

In summary, the present studies demonstrate that VP, or at least its V₂ moiety, in addition to its well-known effects on urinary concentrating activity, also induces an increase in urinary albumin excretion in rats and humans. This effect is probably of glomerular origin, seems to depend on the effects of the hormone on its renal and/or extra-renal V₂ receptors and to involve, at least for part of it, the renin–angiotensin system. More information is needed to fully elucidate the mechanism of this novel effect. Together with previously published observations, these results also suggest that VP, known to be elevated in diabetes mellitus, may contribute to the albuminuria often observed in this disease.

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