Diabetes-induced albuminuria: role of antidiuretic hormone as revealed by chronic V2 receptor antagonism in rats

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Abstract

Background. Vasopressin, an antidiuretic hormone, is elevated in diabetes mellitus (DM). The aim of this study was to evaluate whether the V2 receptor-mediated actions of vasopressin contribute to the albuminuria of diabetes.

Methods. Fourteen adult male Wistar rats with streptozotocin-induced DM were treated over 9 weeks with a selective, non-peptide, orally active V2 receptor antagonist (SR 121463) and were compared to 14 untreated diabetic rats (control). The dose of antagonist was adapted in order to maintain urine osmolality close to plasma osmolality, but not to induce the formation of hypoosmotic urine. Every second week, urine was collected in metabolic cages for two 24h periods.

Results. Urinary albumin excretion (UAE) rose regularly and significantly with time in the untreated control group, whereas it did not rise in treated rats. Interestingly, a variable pattern of UAE increase over time was observed in different rats of the control group. Some rats exhibited pronounced progression of albuminuria with time, while others showed no or only a very modest rise. An a posteriori partition of the control group into ‘progressors’ and ‘non-progressors’ revealed that progressors had more intense urinary concentrating activity, higher creatinine clearance and larger relative glomerular mesangial area than the other subgroup.

Conclusions. This study shows that V2 receptor-mediated actions of vasopressin play a critical role in the albuminuria of diabetes. It also reveals that individual rats, like humans, seem to exhibit an unequal susceptibility to diabetic nephropathy, or at least to albuminuria, a factor considered to be one of its early manifestations.

Keywords: diabetes mellitus; free water clearance; glomerular filtration rate; mesangium; urinary albumin excretion; vasopressin

Introduction

An elevation in plasma vasopressin is well documented in patients with either type I or type II diabetes mellitus (DM). This elevation also occurs in animal models. Rats with streptozotocin-induced DM exhibit a 2- to 7-fold elevation in plasma vasopressin, and BB rats with genetic diabetes exhibit an even greater elevation [1,2]. Recent studies have shown that this elevation contributes to limit the rise in urine output accompanying the markedly increased solute excretion resulting from glycosuria and the increased food intake and protein catabolism [3,4]. However, a study performed in Brattleboro rats genetically devoid of vasopressin suggested that, besides this beneficial effect, the rise in vasopressin could also represent a risk factor for diabetic nephropathy: hyperfiltration, microumbilinuric and renal hypertrophy were blunt or absent 2 and 4 weeks after induction of DM by streptozotocin in these rats compared to their vasopressin-replete Long–Evans controls [4].

Previous studies have shown that the V2 receptor-mediated antidiuretic activity of vasopressin is responsible for a rise in glomerular filtration rate (GFR) in normal rats [5] and for a more rapid progression or renal failure, including increased proteinuria, tubulointerstitial injury and glomerulosclerosis, in several rat models [6–8]. Moreover, dDAVP, a selective V2 agonist of vasopressin devoid of pressor effect, has been shown to induce an increase in urinary albumin excretion (UAE) in normal rats and healthy humans, but not in patients with nephrogenic diabetes insipidus, lacking functional V2 receptors [9].

The present study was designed to re-evaluate in rats the possible contribution of vasopressin to UAE, a
feature considered to be an early sign of diabetic nephropathy, and more specifically to determine whether this effect depends on the V₂ receptor-mediated antidiuretic activity of this hormone. With this aim, a chronic treatment with a selective, non-peptide V₂ receptor antagonist was applied for >2 months to rats with streptozotocin-induced DM. This treatment was intended to suppress the concentrating activity of the kidney, but not to induce the production of dilute urine. Renal function and morphology of treated diabetic rats were compared to those of untreated diabetic rats. In addition to this group comparison, intended to reveal the possible role of V₂ receptor-dependent actions on diabetes-induced albumin excretion, the relatively large number of rats included in this study made possible an evaluation of interindividual variability in the development of albuminuria in untreated rats.

Materials and methods

Experimental protocol

The study was performed in 36 adult male Wistar rats [initial body weight (FW) 230–240 g] purchased from Iffa Credo (L’Arbresle, France). Rats had free access to tap-water and standard food (A03, UAR, Epinay/Orge, France) during the whole study. In 28 of the rats, DM was induced by one (or occasionally two) i.p. injection(s) of streptozotocin (65 mg/kg BW) (Sigma) in a 2% solution of 0.1 M sodium citrate buffer (pH 4.5). Diabetes was confirmed by the appearance of glucose in urine (Keto-diabur tips, Boehringer-Mannheim, Mannheim, Germany). The other eight rats were injected with 300 µl of sodium citrate buffer alone and served as non-diabetic controls (Non-DM group).

Nine days after induction of DM, all rats were placed in individual metabolic cages where they remained for the rest of the study. After 5 days of adaptation, two consecutive 24 h urine collections were performed in order to establish basal values for all parameters including BW, food and fluid intake, urine flow rate (V), urine osmolality (Uosm), and urinary concentrations of albumin, glucose and creatinine. After completion of urine collections, a blood sample (800 µl) was taken from a jugular vein in a heparanized tube under general anaesthesia (i.p., ketamine + xylazine, 5.8 and 0.54 mg/100 g BW, respectively) for measurement of plasma concentration of glucose, sodium and creatinine.

DM rats were then divided into two groups of 14 rats each, with equivalent UAE, urine concentrating activity (V and Uosm), osmolar excretion, plasma glucose concentration (Pglu) and BW. One week later (i.e. after 3 weeks of DM), treatment with the V₂ antagonist was initiated in one of the groups (DM-SR), whereas no intervention was made in the other group (DM-Cont). This treatment was continued for 9 weeks. Chronic V₂ antagonism was achieved in DM-SR rats by oral administration of the non-peptide V₂ receptor antagonist SR 121463B (referred to further as SR or anti-V₂) (Sanofi Synthélabo, Toulouse, France). The drug was added to powdered food mixed with a small amount of water (0.5 ml/g food), and the mixture was provided ad libitum. The concentration of drug in the food was adjusted weekly in each rat in order to counteract the concentrating activity of the kidney without inducing the formation of hyposmotic urine, i.e. to bring Uosm into the 300–400 mosmol/kg H₂O range. This was achieved with 15–50 mg/kg anti-V₂ per day.

Every second week after initiation of the anti-V₂ treatment, urine was collected for two 24 h periods, a blood sample was taken, and the same measurements as in the basal week were repeated (see above). In addition, on the seventh and eleventh weeks of DM, systolic blood pressure (SBP) was measured by the tail-cuff method (BP recorder 8005, Apelex, Massy, France) on three successive days per week, and results of the last 2 days of each week were averaged for each rat.

After the last urine collection (on the twelfth week of diabetes), rats were anaesthetized as described above and a last blood sample was taken. Kidneys, liver and heart (without auricles) were removed and weighed. The left kidney was fixed by immersion in 5 ml of Bouin’s solution for subsequent histological analysis.

Biochemical analyses

Osmolality was measured with a freezing-point microsometer (Roebling, Berlin, Germany). Sodium concentration was measured with a flame photometer (IL-243, Instrument Laboratory, Paris, France) and glucose concentration with the Glucose Trinder kit (Sigma). Urinary albumin concentration was measured by radial immunodiffusion using a rabbit antiserum against rat albumin (Nordic Immunology, Tilburg, The Netherlands). Creatinine concentration was determined with an enzymatic assay (single-slide method, Bioxyzer, Kodack, Johnson and Johnson, USA). Daily excretion of total osmole, glucose, albumin and creatinine, as well as creatinine clearance (Ccreatin) (an index of GFR) and solute-free water reabsorption (TFH₂O), were calculated according to standard formulas.

Glomerular morphometry

Fixed kidneys were embedded in paraffin and cut in 4-µm-thick transversal sections. Sections were stained with Masson’s trichrome for qualitative histology and silver staining for morphometry and observed under light microscopy. For each rat, quantitative morphometric analysis of 60 randomly sampled glomeruli (30 in the superficial cortex and 30 in the juxtamedullary cortex) was performed with an automated computerized image analysis system, as described previously [10]. All glomeruli were measured with a ×40 objective giving a final calibration of 0.412 µm/pixel. For each glomerulus, measurements included total glomerular surface area (limited by the internal edge of Bowman’s capsule), glomerular tuft surface area, total area of capillary lumens and capillary-free walls, and total urinary space surface area [10]. These measurements allowed the calculation of the relative mesangial area (expressed as a percentage of the whole glomerular cross-sectional surface area).
Statistics

All urine data are means of two successive 24h urine collections for each rat. Results are expressed as group means ± SEM. Data obtained in Non-DM rats are shown only as reference and were not considered in the statistical analysis. The influence of the anti-V₂ treatment on different variables along the course of the study was assessed by one-way ANOVA with repetition, followed by Fisher’s post hoc test (or Dunnett’s test for data shown in Figure 2). Organ weights were compared by Student’s t-test. Differences between ‘progressors’ and ‘non-progressors’ in the DM-Cont group were analysed by Student’s t-test.

Results

Streptozotocin injection resulted in the usual rise in \( P_{\text{glu}} \) (up to 33–35 mmol/l) and in the appearance of significant glycosuria (80 mmol/d). As designed in the experimental protocol, both DM groups exhibited similar values for these two parameters upon initiation of the anti-V₂ treatment, indicating a similar severity of DM. Rats received no insulin treatment during the study and their \( P_{\text{glu}} \) remained stable during the whole study (Figure 1).

Eight and 10 weeks after induction of diabetes, three out of the 28 DM rats died shortly after anaesthesia and blood sampling (two DM-SR and one DM-Cont). Measurements made in this last blood sample revealed marked disturbances in water balance (very high plasma osmolality and plasma urea concentration). Accordingly, values for these three rats (concerning urine and plasma) obtained during the last week before death were not included in the means.

The diuretic effect of the V₂ antagonist in DM rats is illustrated in Figure 2. By weekly adjustment of the

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**Fig. 1.** Physiological parameters observed in non-diabetic rats (Non-DM) and in diabetic rats untreated (DM-Cont) or treated (DM-SR) with the vasopressin V₂ antagonist. Open bars show results obtained in the basal period, before the anti-V₂ treatment, and hatched bars show the results obtained after 9 weeks. BW, body weight; \( P_{\text{glu}} \), plasma glucose concentration; \( E_{\text{creat}} \), \( E_{\text{glu}} \) and \( E_{\text{osm}} \), creatinine, glucose and osmolar excretions, respectively; UAE, urinary albumin excretion. Results are means ± SEM. ANOVA with repeated measures followed by Fisher’s post hoc test. Time comparison: ***\( P < 0.001 \); **\( P < 0.01 \); *\( P < 0.05 \) vs basal week in the same group.
anti-V2 treatment in each rat, Uosm was kept close to 400 mosmol/kg H2O in DM-SR rats, as compared to values of ∼1000 mosmol/kg H2O in DM-Cont rats. Conversely, V was markedly higher in DM-SR rats than in DM-Cont rats (300–350 vs 150 ml/d). Plasma sodium concentration increased moderately and non-significantly in response to the anti-V2 treatment (from 132 ± 1 to 135 ± 1 mmol/l). These values were maintained for the 9 weeks of treatment. Interestingly, in DM-SR rats, a significant positive correlation was observed between the dose of antagonist required to attain the target Uosm and the daily osmolar excretion (data-points for all DM-SR rats on weeks 3, 5 and 7 of treatment, r = 0.424, P < 0.01).

The V2 antagonist had no effect on food intake, which remained similar in the two groups (but distinctly higher in DM rats than in Non-DM rats, as already well known). Food intake on week 9 was 21.2 ± 0.6, 38.0 ± 1.0 and 36.7 ± 0.8 g/d in Non-DM, DM-Cont and DM-SR rats, respectively. Accordingly, daily osmolar excretion was also similar in DM-Cont and DM-SR rats, in the basal week and after 9 weeks of treatment. Glucose excretion was also not influenced by the treatment (Figure 1). As already reported [3,4], T'H2O was markedly higher in DM rats than in Non-DM rats (300 ml/d in DM vs 75 ml/d in Non-DM). As intended, the V2 antagonist almost completely suppressed T'H2O in DM-SR rats (2 ± 12 vs 280 ± 13 ml/d in DM-Cont, at 9 weeks, P < 0.001).

The well-known hyperfiltration of diabetes was present in DM rats. During the basal week, Ccreat amounted to 1.29 ± 0.05 and 1.32 ± 0.08 ml/d per 100 g BW in the two DM groups vs 0.97 ± 0.07 ml/d per 100 g BW in the Non-DM group. Ccreat expressed per unit BW tended to decrease with time, the fall being equivalent in the two DM groups and the Non-DM group (amounting to ∼20% in 10 weeks). No effect of the anti-V2 treatment was observed on Ccreat, whether in absolute terms or relative to BW. However, GFR may actually have been lower in DM-SR rats than in DM-Cont rats, as will be explained in the Discussion.

Blood pressure was not influenced by the V2 antagonist: SBP was 146 ± 2 mmHg in Non-DM rats, 148 ± 4 in DM-Cont rats and 145 ± 3 in DM-SR rats 4 weeks after initiation of the anti-V2 treatment; it was 149 ± 4, 153 ± 4 and 148 ± 3 mmHg, respectively, after 9 weeks (not significant).

During the basal week, UAE, but not creatinine excretion, was higher in DM rats than in Non-DM rats, as shown in Figure 1. Albuminuria rose progressively with time in DM-Cont rats. In contrast, albuminuria remained stable and equal to basal values in DM-SR rats (Figure 2). As a result, UAE was twice as high in DM-Cont rats than in DM-SR rats after 9 weeks of treatment (Figures 1 and 2). In contrast to albumin excretion, creatinine excretion was similar in all DM rats and was not affected by the V2 antagonist treatment (Figure 1).

Figure 3 depicts the relationships observed among individual rats between Ccreat or UAE and TcH2O, the main index of urinary concentrating activity. Significant positive correlations were observed in DM-Cont rats, suggesting that a higher concentrating activity was associated with higher GFR and albuminuria. A significant positive correlation was also observed in DM-Cont rats between Ccreat and UAE (data not shown, r = 0.809, P < 0.001). In contrast, no correlation was present in DM-SR rats, and values observed in this group were close to those of the Non-DM group (Figure 3).

Figure 3 also reveals a marked interindividual variability in UAE among DM-Cont rats. Moreover, two out of the 13 DM-Cont rats exhibited a much higher UAE during the whole study than the other rats of the same group, reaching values >12 mg/d during the last week (Figure 3). No such heterogeneity was observed in DM-SR rats. A single rat in this group suddenly exhibited a high UAE (15 mg/d) on the very last week of treatment, but UAE in this rat had been in
the same range as in the other 13 DM-SR rats during all preceding weeks.

Body and organ weights at the end of the study are shown in Table 1. DM rats were lighter than Non-DM rats, as already known. The V2 antagonist treatment induced no significant difference in BW. Both kidney and liver, but not heart, exhibited a distinct hypertrophy as a consequence of DM (the DM-Cont/Non-DM ratio for organ weight per 100 g BW = 1.75 for kidney, 1.46 for liver and 1.07 for heart). Treatment by the V2 antagonist resulted in a slight reduction in the absolute weight of the three organs (Table 1). For liver and heart, these differences were proportional to the small difference in BW and thus were abolished when the weight of these two organs was expressed relative to BW. In contrast, the difference observed for the kidney remained almost significant even relative to BW, thus suggesting that the V2 antagonist reduced

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**Table 1.** Body and organ weights observed at the end of the study in non-diabetic rats (Non-DM) and in diabetic rats untreated (DM-Cont) or treated with the vasopressin V2 antagonist (DM-SR)

<table>
<thead>
<tr>
<th></th>
<th>Non-DM</th>
<th>DM-Cont</th>
<th>DM-SR</th>
<th>t-testa</th>
<th>DM-SR/DM-Cont</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>8</td>
<td>13</td>
<td>12</td>
<td>NS</td>
<td>0.93</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>444±12</td>
<td>324±12</td>
<td>301±12</td>
<td>NS</td>
<td>0.93</td>
</tr>
<tr>
<td>Kidney weight (g)</td>
<td>2.34±0.11</td>
<td>3.02±0.13</td>
<td>2.47±0.10</td>
<td>P&lt;0.01</td>
<td>0.82</td>
</tr>
<tr>
<td>Kidney weight (g/100 g BW)</td>
<td>0.53±0.02</td>
<td>0.93±0.03</td>
<td>0.83±0.04</td>
<td>P=0.06</td>
<td>0.89</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>12.1±0.4</td>
<td>12.9±0.29</td>
<td>12.0±0.30</td>
<td>P&lt;0.05</td>
<td>0.93</td>
</tr>
<tr>
<td>Liver weight (g/100 g BW)</td>
<td>2.73±0.05</td>
<td>3.98±0.04</td>
<td>4.02±0.14</td>
<td>NS</td>
<td>1.01</td>
</tr>
<tr>
<td>Heart weight (mg)</td>
<td>980±10</td>
<td>764±18</td>
<td>693±20</td>
<td>P&lt;0.05</td>
<td>0.91</td>
</tr>
<tr>
<td>Heart weight (mg/100 g BW)</td>
<td>221±6</td>
<td>236±5</td>
<td>232±8</td>
<td>NS</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Results are means ± SEM. NS, not significant.

aStudent’s t-test between DM-SR and DM-Cont.
diabetes-induced kidney hypertrophy compared to DM-Cont rats.

Kidney histology revealed no glomerular lesions in either group of rats. The morphometric study also did not reveal any difference between DM-Cont and DM-SR rats in what concerns glomerular cross-sectional surface area, capillary surface area, mesangial surface area or relative mesangial area in either superficial or deep glomeruli (data not shown). Relative mesangial area was larger in deep glomeruli than in superficial glomeruli, by 25% in Non-DM rats \((P < 0.05)\) and by 37% and 35% in DM-Cont and DM-SR rats, respectively \((P < 0.01\) for both).

Interestingly, positive and significant correlations were observed among 11 DM-Cont rats (two rats with UAE >2 mg/d excluded; Figure 3) between the relative mesangial surface area in superficial glomeruli and the intensity of albuminuria \((r = 0.784, P < 0.01)\) or the level of \(C_{\text{creat}}\) \((r = 0.662, P < 0.05)\). Correlations were not significant in deep glomeruli, but these represent only \((~15\%\) of all glomeruli in the kidney and thus contribute to only a relatively small fraction of total UAE and \(C_{\text{creat}}\). No such correlations were observed in either superficial or deep glomeruli of DM-SR rats.

A large interindividual variability in UAE was present among DM-Cont rats, as revealed by large SEMs in Figure 2 and by individual data in Figure 3. Analysis of individual values over time showed two different patterns. In half of the rats, UAE did not rise at all in 10 weeks, while in others, a significant and progressive rise was observed, leading to 1.5- to 5-fold higher values on week 9 than on the basal week. Thus, UAE ‘progressed’ in some rats and did not in others, in spite of the fact that none of these rats received any treatment. Figure 4 shows an a posteriori partition of the DM-Cont group into ‘progressors’ (DM-Cont-P) and ‘non-progressors’ (DM-Cont-NP). No difference in UAE existed between these two subgroups in the urine data preceding the onset of the treatment (basal). Note that UAE in DM-Cont-NP at week 9 was not different from UAE in DM-SR rats \((1.98 \pm 0.26\, \text{mg/d in DM-Cont-NP vs } 1.82 \pm 0.11\, \text{mg/d in DM-SR})\).

Based on this a posteriori distinction between two subgroups of DM-Cont rats, we further analysed other parameters that could be associated with UAE. Table 2 shows that several significant differences were observed between DM-Cont-P and DM-Cont-NP, including higher BW, \(C_{\text{creat}}\), \(V\), \(T^2\text{H}_2\text{O}\) and osmolar and glucose excretion in DM-Cont-P than in DM-Cont-NP. Note that values observed in DM-Cont-NP are very close to those observed in DM-SR rats. The most striking difference between DM-Cont-P and DM-Cont-NP was the difference in \(C_{\text{creat}}\), which persisted even when \(C_{\text{creat}}\) was factored by BW (26% higher in DM-Cont-P than in DM-Cont-NP) (Table 2). Associated with these functional differences, a significant mesangial expansion was present in DM-Cont-P compared to DM-Cont-NP, in both superficial and deep glomeruli. The proportion of the whole glomerular cross-sectional surface area occupied by the mesangium was on the average 1.4 times larger in DM-Cont-P than in DM-Cont-NP (Table 2).

Discussion

Because vasopressin is known to be elevated in DM [2], because vasopressin V2 agonism has been shown to increase UAE in normal rats and healthy humans [9] and because no increase in albuminuria with time
occurred in DM rats lacking this hormone [4], the present study investigated the influence of chronic vasopressin V2 antagonism on renal function and morphology in rats with experimental type I DM. This study brings three new results. First, it reveals that diabetes-induced microalbuminuria is totally prevented by chronic antagonism of the V2-mediated actions of vasopressin for several weeks. Secondly, it suggests that interindividual differences in albuminuria (and hence probably in susceptibility to diabetic nephropathy), well documented in humans, are also present in rats. Thirdly, it shows that microalbuminuria and GFR in DM rats are positively correlated to the intensity of the kidney’s concentrating activity.

Use of a V2 antagonist in rats with DM

Previous studies have revealed that the influence of vasopressin and/or urinary concentrating activity on GFR in normal rats and in humans is restricted to the hyperosmolar range, i.e. to situations in which \( U_{\text{osm}} \) is higher than plasma osmolality. This influence is no longer observed during the formation of hypoposmotic urine (see Figure 9 in Bankir [11]). For this reason, it seemed appropriate in the present study to counteract the concentrating activity of the diabetic kidney, but not to induce the formation of hypotonic urine. Because of interindividual variability, the dose of antagonist needed to achieve this goal was not the same in all rats, but was relatively stable in each rat. We observed, a posteriori, that the dose of antagonist required for maintaining the target \( U_{\text{osm}} \) was positively correlated with the daily excretion of osmolites. This suggests a link between the endogenous level of vasopressin and the solute load that the kidney needs to concentrate. Note also that the doses required to lower \( U_{\text{osm}} \) to the target level in DM rats were several times higher than those needed in Non-DM rats (15-50 vs 3 mg/kg) [12]. This difference is also most probably related to the markedly increased solute load (~120 mosmol/d in DM vs ~25 mosmol/d in Non-DM) and the rise in plasma vasopressin that occurs in DM [2].

During the anti-V2 treatment, the increased urine output was balanced by an equivalent increase in fluid intake, so that rats were not significantly dehydrated (no significant change in plasma sodium). The anti-V2 treatment did not influence BW, food intake and osmolar excretion. It also did not affect glycaemia and glycosuria. These observations suggest that V2-mediated actions of vasopressin do not participate in the increased appetite and metabolic derangements characteristic of DM, as already discussed [4].

Effect of V2 antagonism on albuminuria of rats with DM

The most striking result of this study is the absence of a progressive rise in diabetes-induced albuminuria observed during chronic blockade of V2 receptors. Albuminuria in DM-SR rats remained at its initial level right from the first week of treatment, whereas it increased steadily for 9 weeks in DM-Cont rats. GFR tends to increase when urinary concentrating activity is stimulated acutely or chronically [13]. A significant positive correlation has been documented between GFR and \( T^2 H_2O \) in normal rats [5,11] and in humans [14]. In the DM-Cont rats in the present study, both GFR and albuminuria were positively correlated with \( T^2 H_2O \). Note that, in these rats, the range of
$^{3}$H$_2$O was much higher than in Non-DM rats (200–300 vs 10–60 ml/d).

Several studies have revealed a lower GFR and kidney weight in rats in which urinary concentrating activity was chronically reduced [5,15], so it was logical to assume that GFR could be lower in DM-SR rats than in DM-Cont rats in the present study. This did not seem to be the case, because $C_{\text{crea}}$ was similar in both groups. However, $C_{\text{crea}}$ tends to overestimate GFR in situations involving a high $P$ [5,16,17]. Accordingly, it may be assumed that DM-SR rats actually had a lower GFR [15]. They also exhibited a 11% lower kidney weight/BW ratio than DM-Cont rats.

The mechanism by which the intensity of urinary concentrating activity could influence renal haemodynamics and albuminuria cannot be inferred from the present study. It does not result from a reduction in blood pressure, as could have been the case in previous studies [6,18], because the anti-V$_2$ treatment did not influence SBP in DM rats. Moreover, acute dDAVP infusion has been shown to increase UAE in healthy humans during a fall in blood pressure, and chronic infusion of dDAVP in rats has been shown to increase UAE without influencing blood pressure [9].

A direct effect on the glomerular filter does not seem likely because no V$_2$ receptors or corresponding mRNA have been identified in glomeruli, even with sensitive methods like RT–PCR [19]. As explained elsewhere [2,5], an indirect effect could implicate the renin–angiotensin system because studies in normal rats have shown that the albuminuric effect of dDAVP was reduced during chronic blockade of this system [9].

In the aggregate, these observations suggest that the anti-V$_2$ treatment reduced glomerular hyperfiltration and kidney hypertrophy and prevented the rise in UAE usually associated with DM [4]. In the present study, effects of vasopressin mediated by V$_1$ receptors were not abolished. They may even have been increased because chronic blockade of V$_2$ receptors is known to increase endogenous vasopressin secretion [18,20,21]. Thus, the present study demonstrates a predominant involvement of V$_2$-mediated actions of vasopressin in the albuminuria of DM.

A limitation of this study is its relatively short duration, which did not allow overt diabetic nephropathy to become apparent. DM-Cont rats did not develop detectable glomerulosclerosis, and $C_{\text{crea}}$ remained higher in DM rats than in Non-DM rats during the whole study, thus showing no decline in renal function. In humans, albuminuria also occurs before renal function declines, and it is known to be an early sign of subsequent renal failure. It is usually thought that rats exhibit a much higher basal albumin excretion than humans, but this difference is no longer apparent when UAE is factorized by GFR, and it likely parallels allometric differences in food intake, osmolar excretion and GFR. In both species, the fraction of plasma albumin filtered through the glomeruli is ~0.1% [22,23]. Thus, we may assume that the changes in albumin excretion reported in the present study have the same pathophysiological significance as the appearance of microalbuminuria in humans.

Thus, although this study is too short to document a significant role of V$_2$-mediated actions of vasopressin in overt diabetic nephropathy, it strongly suggests an involvement of this hormone in the early signs of its manifestation. This possibility is consistent with the fact that dDAVP, a V$_2$ agonist, increases UAE even in normal rats and healthy humans, except in those who exhibit loss-of-function mutations of the V$_2$ receptor [9].

**Variable susceptibility of rats with DM to develop microalbuminuria**

Another interesting result of this study is the wide intrar species variability in the evolution of UAE over time in DM-Cont rats: UAE rose in only 7 out of 13 rats. In humans, microalbuminuria also occurs in only a fraction of diabetic patients and is usually followed within a few years by more severe renal dysfunction. The present observation thus suggests that rats may exhibit different individual susceptibility to diabetic nephropathy, as do humans. This susceptibility is probably genetically determined [24]. The rat strain used in this study (Wistar) is not an inbred strain, and thus different rats may exhibit significant genetic variability. Moreover, UAE was significantly correlated with hyperfiltration, and both parameters were also positively correlated with the intensity of the concentrating effort of the kidney ($^{3}$H$_2$O).

Other factors also differed between the rats of the two subgroups (Progressors and Non-Progressors). Progressors exhibited higher $C_{\text{crea}}$, larger relative mesangial surface area, higher glucose and total osmolar excretions and a higher $^{3}$H$_2$O, the latter suggesting a greater urinary concentrating effort. All these differences largely exceed the small difference in BW (the Progressors/Non-Progressors ratio ranging from 1.27 to 1.47 for functional and anatomical parameters vs only 1.09 for BW; Table 2). Notably, the mesangial surface area was significantly correlated with $C_{\text{crea}}$ and UAE. In humans, mesangial expansion and glomerulosclerosis are usually preceded by an increase in albuminuria [25]. In the aggregate, observations made in DM-Cont rats in the present study reveal a good agreement between several signs associated with diabetic nephropathy (increases in GFR, in UAE and in mesangial surface area) and a link between these signs and the concentrating activity of the kidney ($^{3}$H$_2$O).

In conclusion, the use of a highly selective, nonpeptide, orally active V$_2$ antagonist in DM rats for 9 weeks established the critical role played by the antidiuretic effects of vasopressin in the early rise in UAE associated with DM. Further studies are required to elucidate the mechanism responsible for this effect. This study also suggests that the rat kidney, like that in humans, exhibits individual variability in its response to the perturbations induced by this metabolic disease.
Vasopressin V2 receptor antagonism in diabetes mellitus

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