With diabetes mellitus, the ability of the kidneys to maintain fluid balance is affected. Hyperglycaemia increases production of hyaluronan in cultured kidney cells implying that diabetes promotes induction of hyaluronan in the kidney. The aim of the present study was to determine if the interstitial matrix component hyaluronan is differently distributed within the kidney in diabetic rats compared to non-diabetic rats. Furthermore, to test if diabetic rats are able to respond with diuresis upon a hypotonic fluid load. The normal heterogeneous intrarenal distribution of hyaluronan was confirmed in non-diabetic control rats, with 60-fold more in the papilla than in the cortex. In diabetic animals, the cortical hyaluronan was unaffected but the papillary hyaluronan content was 3-fold higher than in non-diabetic rats. This increase correlated with a more than three-fold induction of the papillary hyaluronan-synthase 2 mRNA expression. In non-diabetic animals, 2 h water loading increased papillary hyaluronan (+93%) and diuresis (17-fold). In diabetic animals, baseline diuresis was 8-fold higher than in non-diabetic animals, which correlated with hyperglycaemia, glucosuria and proteinuria. Water loading in diabetic animals did not further increase papillary hyaluronan or diuresis: the urine flow rate decreased. To conclude, papillary hyaluronan is elevated in diabetic rats, which coincides with induction of hyaluronan-synthase 2 mRNA, hyperglycaemia, glucosuria, proteinuria and overt diuresis. The inability to respond to a water load with further diuresis may be related to the already elevated papillary hyaluronan and the inability to change hyaluronan during water loading.

Key words: diabetes, hyaluronan, kidney, water loading, diuresis

INTRODUCTION

Patients and rats with uncontrolled type 1 diabetes mellitus often have osmotic diuresis due to unabsorbed glucose in the tubule lumen that can result in
volume depletion. The diuretic/natriuretic response in diabetic rats to isotonic volume expansion is attenuated and an increased sympathetic nerve activity may play a role (1). Furthermore, the abundance of papillary key proteins for urinary concentration, such as aquaporin 2 (AQP2) and urea transporter A1 (UT-A1), is elevated in spite of ongoing osmotic diuresis (2). The plasma level of vasopressin is elevated during diabetes (3, 4) and it is unknown whether the renal response to hypotonic fluid loading, i.e. the ability to produce water diuresis, is also abrogated.

Previous studies (5 - 8) demonstrate an involvement of the interstitial matrix component hyaluronan (HA) in renal water handling. This connective tissue polysaccharide has large water binding effects due to its negative charge (9) and during normal physiological conditions it is found primarily in the renal papilla, and scarcely in the cortex (7, 8, 10). Together with vasopressin-regulated AQP (11) the papillary interstitial content of HA changes in relation to the body water content i.e. during water diuresis and dehydration (7, 8), and determine papillary water permeability. It has also been suggested that the inability of the immature kidney to concentrate urine is dependent on high interstitial HA (12). Furthermore, during pathological conditions of renal function, such as renal failure, tubulointerstitial fibrosis, and renal transplant rejection, the cortical amount of HA increases, which gives rise to interstitial inflammation and oedema with consequences for water transport properties (10, 13). During hyperglycaemia, several kidney cells (proximal tubular cells, renal fibroblasts, mesangial cells, glomeruli) in culture produce HA at an increased rate (14 - 17), implying that diabetes promotes induction of HA in the kidney, which may be involved in the development of diabetic nephropathy due to changes in matrix composition and properties that affect function.

The present study on uncontrolled streptozotocin (STZ)-induced diabetic rats investigated if HA was differently distributed in the kidney in vivo than in non-diabetic control rats. To elucidate the possible origin of such a change, the mRNA levels of HAS2, a main HA synthase, was analysed. To determine a possible consequence, the ability of these rats to produce diuresis in response to a hypotonic fluid load was also tested.

MATERIALS AND METHODS

All experimental procedures were conducted in accordance with guidelines of the Swedish National Board for Laboratory Animals and were previously accepted by the regional ethics committee for animal experiments. The experiments were performed on 49 male Wistar Furth rats, body weight (bw) 290 - 325g (B&K Universal, Sollentuna, Sweden).

All animals were allowed free access to standardized chow (R3, Ewos, Södertälje, Sweden) and tap water. The pellet food contained 3g·kg⁻¹ Na⁺, 8g·kg⁻¹ K⁺ and 21% protein. Three animals were housed per Macrolone type IV cage (Techniplast, Buguggia, Italy). The rats were kept in standard
animal rooms under standard animal housing conditions: lighting regime 12 h light, 12 h darkness, temperature 21 ± 1°C and relative humidity 30-60%.

**Induction of diabetes**

The animals were rendered diabetic by a single injection into the tail vein of STZ (45mg·kg⁻¹ bw, Sigma-Aldrich, St. Louis, Missouri, USA) dissolved in isotonic Ringer® solution (Fresenius Kabi, Halden, Norway). Seven days after the injection, and on the day of the surgery, the tip of the tail was cut and the blood glucose level was determined with test reagent strips (Medisense® Blood glucose sensor electrode, Abbott Laboratories, Bedford, UK). The animals were considered diabetic if the blood glucose level was ≥20mM. Four weeks elapsed between the induction of diabetes and the experiments. The non-diabetic control animals received a sham injection of isotonic Ringer® solution.

**Anaesthesia and surgical procedures**

The animals were anaesthetized with an intraperitoneal injection of Inactin® [5-ethyl-5-(1-methyl-propyl)-2-thiobarbiturate sodium] (Sigma-Aldrich, St Louis, Missouri, USA) 120 mg·kg⁻¹ bw for non-diabetic animals and 80 mg·kg⁻¹ bw for diabetic animals.

The animals were then placed on a servo-controlled heating pad in order to maintain a temperature of 37.5°C throughout the surgery. Tracheotomy was preformed to maintain free breathing and the left femoral artery was catheterised for continuous measurement of mean arterial blood pressure (MAP) (Statham P23Dc, Statham Laboratories, Los Angeles, CA, USA) and for blood sampling. The left femoral vein was catheterised for substance administration and for continuous infusion of saline. The urinary bladder was catheterised through a suprapubic incision for urine sampling.

**Experimental protocols in vivo**

After the surgical procedures, all animals were allowed to recover for 45 minutes before a 30 minute control period, during which an infusion of isotonic Ringer® solution at a rate of 5 ml·h⁻¹·kg⁻¹ bw for non-diabetic animals and at a rate of 10 ml·h⁻¹·kg⁻¹ bw for diabetic animals was administered.

During four consecutive 30-min experimental sampling periods, the rats received either:

1. Isotonic Ringer® solution, as during the control period (non-diabetic 5 ml·h⁻¹·kg⁻¹ bw, diabetic 10 ml·h⁻¹·kg⁻¹ bw), or
2. Hypotonic glucose saline solution (15 ml h⁻¹·kg⁻¹ bw, 100 mOsm·kg⁻¹ H₂O, 0.25 % NaCl, 0.5 % glucose) as water loading (same rate to non-diabetic and diabetic animals).

Urine was collected during all periods. After the experimental procedures, the kidneys were excised, weighed and sectioned into cortex and papilla. The samples were put on filter paper for 3 min and then weighed (wet weight, ww), after which they were frozen and stored at −70°C until analysis for HA content (see below). The animals were euthanised with an intravenous injection of saturated KCl.

**Measurement of hyaluronan and determination of water content**

Frozen specimens were lyophilized overnight and weighed (dry weight, dw). The water content of the tissue in per cent was calculated with the formula: (100 x (ww - dw)/ww). After grinding, HA was extracted from the tissue with 0.5 M NaCl for 16 h, and after centrifugation at 2700 g for 15
min at 4°C: the supernatants were analysed for HA with an enzyme linked immunosorbent assay (ELISA) (Echelon Biosciences Inc., Salt Lake City, Utah, USA).

**HAS2 mRNA isolation**

The excised papillas were stored in RNAlater (QIAGEN, Merck Eurolab, Stockholm, Sweden) before preparation of total RNA, which was isolated with an RNeasy mini kit (QIAGEN, Merck Eurolab, Stockholm, Sweden) according to the manufacturer’s description.

Briefly, papillas were homogenized in buffer RLT. Next, 70% ethanol was added to the homogenates and the samples were mixed and applied to RNeasy mini columns. The homogenates were centrifuged, the flow-through was discarded and the columns were washed with wash buffer RW1. Membrane-bound RNA was treated with DNase I and incubated at room temperature for 15 min. Thereafter the columns were washed. The columns were subsequently washed twice with RPE buffer, and the flow-through discarded after each centrifugation. The RNA was eluted with 30 µl RNase-free water, which was applied to each column twice, and the accumulated flow-through was collected after the centrifugation. RNA was precipitated with 0.3 M NaAc and ice-cold 99% ethanol, and then washed and dissolved in water at a final volume of 10 µl.

**Preparation of cDNA**

Synthesis of cDNA was by Reversed Transcription System (Promega, Madison, WI) and with 9 µl of total RNA per 20 µl cDNA synthesis reaction and 5 mmol/l MgCl₂, 1x Reverse transcription buffer, 1 mmol/l of each dNTP, 1 U/µl Recombinant RNasin® ribonuclease inhibitor, 15 U/µg AMV Reverse Transcriptase and 0.5 µg (dT)15 primer. The RNA and primers were incubated for 5 min at 60°C and all other agents were then added. The reactions were incubated for 60 min at 42°C followed by 5 min at 99°C; they were then stored at -20°C.

**Analysis of HAS2 mRNA expression**

Synthesized cDNA was amplified with the LightCycler (Roche Diagnostics GmbH, Mannheim, Germany). Specific primers were designed by TIB MolBiol, Berlin, Germany. According to the LightCycler protocol 0.5 µl of the cDNA was amplified in a final volume of 10 µl containing 5 µl Fast Start DNA Master SYBR Green (Roche Molecular Biochemicals, Mannheim, Germany) and 0.5 mM of the sense and anti-sense primers (HAS2 - sense GCTCTATGGGGCGTTCCTC, antisense CCAAGATGTAAGTGACTGATTGTCC). The stability of expression of various house-keeping genes, such as TBP (Cybergene AB, Stockholm, Sweden) and G6PDH (TIB Molbiol Syntheselabor, Berlin, Germany) was assessed. The TBP gene was most constant and therefore, for relative quantifications, TBP was used as a reference in the real-time PCR protocol (TBP - sense ACCCTTCCAACATTGACTCTCTATG, antisense ATGATGACTGCGACAAATCGC). The LightCycler Run version 5.32 was used with the following parameters: denaturation at 95°C for 3 min; and amplification with a total of 45 cycles with each cycle, with a denaturation temperature at 95°C for 15 s, annealing temperature at 60°C for 10 s and elongation temperature at 72°C for 15 s.

Controls were included in each run of the real-time PCR assay. For each primer pair, one sample with no cDNA (containing only RNase-free water) was included. Results were analysed for each sample with relative quantification and the difference between sample and control crossing point (cp) values was compared. To render a true value for each mRNA level, the calculated difference was transformed according to $2^{-\Delta\text{cpHas2-cpTBP}}$ to yield the ratio Has2/TBP.
Urine analysis

The urine volumes were measured gravimetrically and the urinary osmolality ($U_{\text{osm}}$) was estimated from the depression of the freezing point (The Fiske® Micro-Osmometer, Model 210, Fiske® Associates, Norwood, Massachusetts, USA). Urinary sodium ($U_{\text{Na}}$) and potassium ($U_{\text{K}}$) concentrations were determined by flame photometry (Flame Photometer IL 543 (Metric), Instrumentation Laboratory, Milano, Italy).

Urine glucose and protein concentrations were determined colorimetrically with an enzymatic assay for glucose (Roche Diagnostics GmbH, Mannheim, Germany) and a DC protein assay for protein (Bio-Rad Laboratories, Hercules, CA, USA).

Histological examination

Non-diabetic control and diabetic animals were euthanised, and the kidneys removed. Tissue was fixed in formalin (4% in PBS) and embedded in paraffin. Embedded tissue blocks were cut into 5µm-thick sections and stained with haematoxylin/eosin, periodic acid-Shiff and Picrosirius for a blinded histopathologic evaluation. The renal tissue was investigated for fibrosis, inflammation, and glomerular and tubular changes.

Statistical analysis

All values are presented as means ± SEM. The data was evaluated with one-way analysis of variance (ANOVA). Comparison between and within groups were performed using Student’s t-test for unpaired or paired comparisons, respectively, with Bonferroni correction. A $p$-value less than 0.05 was considered statistically significant.

RESULTS

Body and kidney weight, blood glucose and haematocrite

The diabetic animals had lower body weight (259 ± 5 g) than the non-diabetic control animals (342 ± 4 g, $p<0.05$) but higher kidney weight (1.27 ± 0.03 g) than the non-diabetic control animals (1.03 ± 0.02 g, $p<0.05$). Blood glucose (BG: 26 ± 0.6 mM) and haematocrite (Hct: 43 ± 0.6%) in diabetic animals was higher than in non-diabetic control animals (BG: 6 ± 0.7 mM, $p<0.05$: Hct: 41 ± 0.7 %, p<0.05) (Table 1).

Urine data and blood pressure

Before water loading, urine flow rate was higher in diabetic animals (22.6 ± 2.7 µl·min$^{-1}$) than in non-diabetic controls (2.6 ± 0.2 µl·min$^{-1}$, $p<0.05$) and urine osmolality was lower (1059 ± 43 mOsm·kg$^{-1}$ H$_2$O) than in the non-diabetic controls (2017 ± 98 mOsm·kg$^{-1}$ H$_2$O, $p<0.05$) (Table 1). Urinary glucose excretion was 56-fold higher in diabetics (1 195 ± 1.0 nmol·min$^{-1}$) than in non-diabetic control rats (21 ± 0.1 nmol·min$^{-1}$, $p<0.05$) and urinary protein excretion was 56% higher in diabetic animals (153 ± 32 µg·min$^{-1}$) than in non-diabetic controls (98 ± 6 µg·min$^{-1}$). Mean arterial blood pressure (MAP) was similar between the groups (diabetic: 116 ± 1 mm Hg: non-diabetic controls: 117 ± 1 mm Hg).
During water loading in non-diabetic control rats, urine flow rate increased 17-fold (Fig. 1) and urine osmolality decreased by 58%. Sodium excretion increased

Table 1. Baseline data in non-diabetic control animals and in diabetic animals. Values are means ± SEM.

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>DIABETES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>342±4</td>
<td>259±5 *</td>
</tr>
<tr>
<td>Kidney weight (g)</td>
<td>1.03±0.02</td>
<td>1.27±0.03 *</td>
</tr>
<tr>
<td>Arterial blood pressure (mm Hg)</td>
<td>117±1</td>
<td>116±1</td>
</tr>
<tr>
<td>Blood glucose (mM)</td>
<td>6±0.7</td>
<td>26±0.6 *</td>
</tr>
<tr>
<td>Haematocrite (%)</td>
<td>41±0.7</td>
<td>43±0.6 *</td>
</tr>
<tr>
<td>Urine flow rate (µl/min)</td>
<td>2.6±0.2</td>
<td>22.6±2.7 *</td>
</tr>
<tr>
<td>Glucose excretion (nmol/min)</td>
<td>21±0.1</td>
<td>1195±1.0 *</td>
</tr>
<tr>
<td>Protein excretion (µg/min)</td>
<td>98±6</td>
<td>153±32 *</td>
</tr>
</tbody>
</table>

*p<0.05 vs. non-diabetic control animals.

Fig. 1. Urine flow rate in non-diabetic control rats (solid lines) and in diabetic animals (dashed lines) during baseline conditions (solid markers) and during 2 h water loading (open markers). * denotes p<0.05 period E4 vs. period C1.
by 77% and potassium excretion more than 2-fold (p<0.05). Blood pressure during water loading remained unchanged (Table 2).

In diabetic animals, the 2 h water loading induced a surprising reduction in urine flow rate from 20.0 ± 2.6 to 12.6 ± 2.2 µl·min⁻¹ (p<0.05) (Figure 1) and urine osmolality increased slightly from 1108 ± 51 to 1238 ± 50 mOsm·kg⁻¹ H₂O (p<0.05). Sodium- and potassium excretion remained unchanged. Blood pressure during water loading also remained unaltered (Table 2).

**HA and HAS 2**

The heterogeneous intrarenal distribution of HA in non-diabetic kidneys was confirmed with small amounts in the cortex (15 ± 3 µg·g⁻¹ dw) and about a 60-

* denotes p<0.05 between E4 and C1.

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**Table 2. Blood pressure and urine data in non-diabetic control animals (CON) and diabetic animals (DIA) without and with (-H2O) 2 h water loading (E1-E4). Each period is 30 min in length. Values are means ± SEM.**

<table>
<thead>
<tr>
<th></th>
<th>C1</th>
<th>E1</th>
<th>E2</th>
<th>E3</th>
<th>E4</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>114±4</td>
<td>118±3</td>
<td>117±5</td>
<td>116±5</td>
<td>111±5</td>
</tr>
<tr>
<td>CON-H2O</td>
<td>115±5</td>
<td>112±3</td>
<td>112±2</td>
<td>109±2</td>
<td>107±3*</td>
</tr>
<tr>
<td>DIA</td>
<td>114±2</td>
<td>114±3</td>
<td>113±4</td>
<td>111±3</td>
<td>108±3</td>
</tr>
<tr>
<td>DIA-H2O</td>
<td>117±3</td>
<td>118±2</td>
<td>116±2</td>
<td>116±3</td>
<td>115±2</td>
</tr>
</tbody>
</table>

| Uosm (mOsm/kg H₂O) |      |      |      |      |      |
| CON   | 2027±228| 2245±160| 2427±175| 2574±213| 2479±172|
| CON-H2O | 2191±224| 2189±196| 1811±263| 1203±246| 924±226*|
| DIA   | 980±103| 1034±102| 1142±116| 1175±105| 1189±85*|
| DIA-H2O | 1092±69| 1081±94| 1187±60| 1225±62| 1247±74*|

| UNaV (µmol/min) |      |      |      |      |      |
| CON   | 0.10±0.02| 0.11±0.02| 0.11±0.02| 0.13±0.03| 0.13±0.02|
| CON-H2O | 0.09±0.03| 0.10±0.04| 0.07±0.02| 0.14±0.05| 0.16±0.03*|
| DIA   | 0.29±0.10| 0.45±0.15| 0.71±0.18| 0.99±0.25| 1.09±0.23*|
| DIA-H2O | 0.24±0.05| 0.26±0.05| 0.32±0.06| 0.26±0.04| 0.21±0.03|

| UKV (µmol/min) |      |      |      |      |      |
| CON   | 0.48±0.13| 0.53±0.14| 0.58±0.10| 0.69±0.09| 0.67±0.08|
| CON-H2O | 0.34±0.05| 0.52±0.09| 0.47±0.10| 1.04±0.45| 1.00±0.19*|
| DIA   | 0.60±0.14| 0.74±0.16| 0.92±0.13| 1.12±0.11| 1.26±0.17*|
| DIA-H2O | 0.66±0.09| 0.71±0.10| 0.69±0.08| 0.52±0.06| 0.53±0.06|
fold higher content in the renal papilla (929 ± 150 µg·g⁻¹ dw) (Fig. 2). In diabetic animals, the cortical content of HA and cortical HAS 2 mRNA expression (Fig. 3) was similar to the non-diabetic controls; whereas, the papillary HA content was 3-fold higher (3217 ± 123 µg·g⁻¹ dw) than in the non-diabetic controls (929 ± 150 µg·g⁻¹ dw, p<0.05) (Fig. 2). The elevated papillary HA in diabetic rats coincided with a three-fold induction of papillary HAS 2 mRNA (Fig. 3).

During water loading in non-diabetic rats the diuretic effect coincided with increased papillary HA content by 93% (from 929 ± 150 to 1790 ± 315 µg·g⁻¹ dw, p<0.05) (Fig. 4) leaving cortical HA unaffected (data not shown). In diabetic animals, the failure to respond to water loading with further diuresis coincided with a failure to further elevate papillary HA (3217 ± 123 vs. 3358 ± 206 µg·g⁻¹ dwt) (Fig. 4).

**Histological examination**

The histological examination of the kidneys did not reveal differences between the non-diabetic and the diabetic kidneys.

**DISCUSSION**

The composition of the interstitial matrix changes during different diseases and the decline in function during diabetic nephropathy correlates with interstitial...
Fig. 3. Renal cortical and papillary hyaluronan synthase 2 (HAS 2) mRNA in non-diabetic control animals and in diabetic animals. Note the logarithmic scale on the ordinate.
* denotes p<0.05 vs. same parameter of non-diabetic control animals.

Fig. 4. Renal papillary hyaluronan content in non-diabetic control animals and in diabetic animals during baseline conditions and after 2 h water loading.
* denotes p<0.05 vs. baseline conditions.
fibrosis (18). HA has both water binding and proinflammatory properties, which would make it a suitable candidate for a matrix component involved in diabetic nephropathy. The present study evaluated the kidney content of the interstitial matrix component HA during uncontrolled diabetes. In this model of diabetes, HA accumulated primarily in the interstitium of the renal papilla; whereas, the cortical content was similar to that in non-diabetic control rats. The induction of papillary HAS2 mRNA suggested that an increased synthesis was primarily responsible for the elevated HA levels and not primarily a reduced degradation by hyaluronidases or abrogated lymph drainage via the cortex. During water loading in diabetic rats, the elevation in papillary HA, which occurs in non-diabetic control rats, was absent i.e. no further elevation was detected and this coincided with an inability to produce an expected diuretic response during the challenge.

The effect of hyperglycaemia on cellular HA production in vitro has previously been demonstrated: renal interstitial fibroblasts (16), proximal tubular cells (15), glomerular (14) and mesangial cells (17) increase production of HA when grown with elevated glucose conditions in the medium. In interstitial fibroblasts, the high glucose have been shown to stimulate HA production through the PKC/TGF-β cascade, whereas, in proximal tubular cells, the HA elevation was associated with NF-kappaβ-activated transcription of HAS2. Whether the same cellular events, leading from hyperglycaemia to increased HA production, are evident in vivo i.e. as in the present study, remains to be elucidated but appears plausible. Other studies demonstrate increased HA content in diabetic kidneys. Malathy and Kurup (19) determined a marginal increase of HA in kidney tissues from diabetic rats and Berenson et al. (20) found increased HA content in some, but not all, kidneys of diseased humans. Wang and Hascall (17) demonstrated accumulation of HA in the glomeruli of diabetic kidneys. The expression of the enzymes responsible for prostaglandin formation (cyclooxygenase -1 and -2) is elevated in the renal papilla during diabetes and PGE₂ formation is increased (21): PGE₂ is a known stimulator of HA production (14, 22).

The elevation of papillary HA during acute hydration in non-diabetic rats has previously been demonstrated (7, 8). The elevation, that occurs within 2 h, coincides with the previously well-described reduction of vasopressin-regulated aquaporins (11), which results in increased water excretion. Recent observations from our laboratory have suggested that nitric oxide (NO) and prostaglandins participate in the processes leading to the elevated HA levels during hydration as both L-NAME and indomethacin can inhibit water-induced elevation of papillary HA (23). Both these hormones regulate fluid- and electrolyte balance by affecting both tubular transport and renal hemodynamics (24). HA has a unique water-binding property and previous physicochemical studies on HA in vitro have shown that the molecules become entangled, forming a network that occupies the solvent space and excludes large molecules when the concentration of high molecular HA exceeds 0.2 mg·ml⁻¹. This phenomenon, steric exclusion, may influence water transport and osmotic activity in the intracellular matrix (25).
the non-diabetic rat, the tissue hydration was highest in the HA-rich papilla with a calculated HA concentration of 0.6 mg·ml⁻¹ water under normal physiological conditions. Thus, the normal accumulation of HA in the papilla should influence the reabsorption of water from the tubules. To enable a diuretic response upon water loading, the relative water reabsorption needs to be decreased in the papillary collecting system. The finding that the papillary HA increased after water loading was in accordance with the hypothesis that HA has a regulatory role in the tubular handling of water. We therefore suggest that decreased numbers of vasopressin-regulated aquaporins (11) together with increased interstitial HA antagonizes water reabsorption. It has also been hypothesized that the limited urinary concentrating performance evident in the immature developing kidney is due to an abundant HA content in the renomedullary interstitium (12). The antagonism of water reabsorption by interstitial HA may involve changes in the interstitial hydrostatic pressure. In accordance with the idea that interstitial HA decreases water transport are the reports by Wang et al. (26, 27) showing that an intraperitoneal injection of HA decreases peritoneal fluid absorption. Furthermore, in the interstitium, HA diminishes the water permeability of the pulmonary membrane (28).

In diabetic rats, the water challenge did not give rise to a further elevation in papillary HA content above the basal level. The underlying mechanism to this failure in response was unknown, but may be inherent to the already severely elevated basal levels. It is obvious that the diabetes-induced levels are much higher than those found in the non-diabetic rats, even during water loading. Therefore, it can be speculated that the diabetic situation with for example hyperglycaemia, increased papillary COX expression (21), and growth factors (29) represent a maximal stimulatory response in the medulla to produce HA. The involvement of the reported elevated hyaluronidase activity in the kidney of diabetic rats (30) may also influence the ability to attain an elevation in interstitial HA during water loading. Whether the reported increased vasopressin level during diabetes (3) contributes to the increased hyaluronidase activity in this model (6), remains to be established.

Whether the elevation in papillary HA during 2 h water loading in non-diabetic control rats is obtained through increased HA synthesis or decreased degradation could not be determined based on the results of the present study; although, the inhibition of prostaglandin synthesis with indomethacin might reduce the ability to elevate papillary HA in response to water loading. As PGE₂ increases HA production (14) and HA synthase activity (22), it can be suggested that part of the response is due to increased production. However, vasopressin may be involved in HA degradation by interaction with hyaluronidases (6), the enzymes responsible for cellular breakdown of HA. During water loading, vasopressin levels are reduced, which theoretically would reduce breakdown leading to elevated HA content. This correlates well with our recent findings that vasopressin reduces the HA content in the supernatant of cultured medullary
interstitial cells (RMIC) as well as in the papilla of rats (31). We have, furthermore, demonstrated that RMIC in culture produces HA at an increasing rate when media osmolality is reduced i.e. simulating a situation of water diuresis, and that the opposite occurs during high media osmolality i.e. during antidiuresis (32). Therefore, the contribution of the elevation in papillary HA during water loading in non-diabetic rats most probably involves both increased synthesis and reduced breakdown of HA.

The response to water loading in diabetic rats in terms of diuresis was different to non-diabetic control rats. Instead of an elevation in diuresis, as demonstrated by non-diabetic control rats, there was a surprising decline in diuresis upon water loading. This inability to respond with diuresis coincided with an inability to further elevate papillary HA. A defective volume regulation in response to isotonic fluid loading during diabetes has been demonstrated and these results extend those findings to include hypotonic fluid loading. These two types of stimuli are not identically sensed nor responded to by the body via the kidneys, as volume and osmolality are sensed and executed through different pathways (33). The diuretic/natriuretic response in diabetic rats to isotonic volume expansion is attenuated and an increased sympathetic nerve activity may be involved (1). Furthermore, the abundance of papillary key proteins for urinary concentration, such as aquaporin 2 and urea transporter A1 (UT-A1), is elevated in spite of ongoing osmotic diuresis (2). The plasma level of vasopressin elevates during diabetes (3, 4). However, it could not be determined in the present study if the already elevated levels of HA, vasopressin, aquaporin 2 and UT-A1 contribute to the inability to produce a diuretic response during a hypotonic fluid load. It is unknown if the already high diuresis, evident during baseline diabetic conditions, interferes with the ability to acutely further elevate diuresis upon hypotonic stimulation. However, it is clear that the response to the water load could achieve a higher diuresis in absolute terms in non-diabetic control rats than the basal high level in diabetic animals.

In conclusion, renopapillary HA is elevated in 4-week STZ-diabetic rats, which coincides with induction of HAS2 mRNA, hyperglycaemia, glucosuria, proteinuria and overt diuresis. The inability of these rats to respond to a water load with further diuresis as in non-diabetic animals may partly be related to the already elevated papillary HA and the inability to change this matrix component during the water loading.

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Abbreviations: MAP: mean arterial blood pressure; Uosm: urine osmolality; \( \text{U}_{\text{Na}}V \): urinary sodium excretion; \( \text{U}_{\text{K}}V \): urinary potassium excretion.
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