Renal sodium handling in experimental diabetes: role of NO

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Abstract Recent studies have suggested that diabetes is a state of increased renal nitric oxide (NO) activity as assessed by urinary excretion of nitrites and nitrates (NOxy), and that NO synthase inhibitors reverse the increased glomerular filtration rate (GFR) observed in experimental diabetes. In addition to being a potent vasodilator in the renal vasculature, NO also plays a role in modulation of renal sodium excretion. To explore the role of NO in diabetes-associated alterations in renal excretory function, renal haemodynamic and sodium handling parameters were evaluated in conscious control (C) and streptozotocin diabetic rats (D) and correlated to the renal activity of NO, as assessed by urinary excretion of its metabolites NOxy. To further explore this issue, the changes in renal haemodynamics and sodium handling were also assessed after NO synthase inhibition with a non-pressor dose of L-nitro-arginine-methyl-ester (L-NAME) and after administration of the NO donor, glyceryl trinitrate (GTN). Systolic blood pressure was not different between C and D rats. D rats exhibited marked hyperglycaemia (P < 0.001), and increases in GFR (P < 0.001), renal plasma flow, filtration fraction, urinary sodium excretion (UNaV, P < 0.001), filtered load of sodium (FLNa, P < 0.01), and a decrease in fractional reabsorption of sodium (FRNa, P < 0.0001). In contrast, total reabsorption of sodium (TRNa) was increased in D rats compared to C rats (P < 0.001). The urinary excretion of NOxy was markedly increased in D rats (P < 0.001). Regression analyses performed in D rats revealed a close relationship between UNaV and GFR and a weaker correlation with urinary NOxy. Although FRNa correlated only with urinary excretion of NOxy, there was a strong relationship between TRNa and GFR. In contrast to D rats, control rats demonstrated only a relationship between TRNa and GFR and no other correlations were found. In D rats, NO inhibition with L-NAME (1 mg/kg body weight) resulted in a marked decrease in GFR and urinary NOxy associated with decreases in FLNa and TRNa but did not influence FRNa. In contrast, in C rats the post-L-NAME decrease in NOxy was not associated with significant changes in GFR and renal sodium handling. GTN-treated C rats exhibited a renal vasodilatory response and an increase in natriuresis and urinary NOxy whereas no renal changes were observed in D rats during GTN administration. The present data indicate that changes in renal sodium handling before and after NO modulation in experimental diabetes are related to changes in GFR rather than to the renal activity of NO. Therefore, in contrast to the effects on renal haemodynamics, NO does not play an important role in the altered renal sodium handling observed in experimental diabetes.

Key words: glyceryl trinitrate; L-nitro-arginine-methyl-ester; nephropathy; nitric oxide; renal sodium handling

Introduction

Several groups including our own have recently suggested that NO is involved in the pathogenesis of renal haemodynamic changes in diabetes [1–3]. Apart from abnormalities in glomerular haemodynamics, early stages of diabetic nephropathy are also associated with alterations in renal sodium handling [4,5]. Despite net increases in urinary sodium excretion (UNaV) [4,6,7] clinical and experimental diabetes are both associated with enhanced tubular sodium reabsorption [5–7]. Changes in tubular sodium handling such as increased sodium–glucose co-transport have been, in addition to other factors, postulated to be implicated in the pathogenesis of renal haemodynamic changes in diabetes [6,7]. Changes in distal sodium delivery may alter activity of the tubuloglomerular feedback (TGF) mechanism with subsequent effects on glomerular haemodynamics [5,8]. Furthermore, enhanced sodium reabsorption may lead to increased cellular sodium content which has been implicated in cell hypertrophy [9], another characteristic feature of diabetic nephropathy. In addition to being a potent vasodilator in the renal vasculature, NO plays an important role in modulation...
of renal sodium excretion [10-14]. It is therefore possible that renal hyperproduction of NO plays a role not only in the pathogenesis of renal haemodynamic changes but also modulates renal excretory functions in diabetes. To explore this possibility, renal haemodynamic and sodium handling parameters were evaluated in conscious control and diabetic hyperfiltering rats and correlated to the renal activity of NO, as assessed by urinary excretion of its stable metabolites nitrites and nitrates (NOx) [15]. It has previously been shown that the elevation in urinary NOx observed in experimental diabetes reflects increased renal rather than systemic NO production [1]. To further explore this issue, the changes in renal hemodynamics and sodium handling were also assessed after NO synthase inhibition with a non-pressor dose of l-nitro-argininemethyl-ester (L-NAME) and after administration of the NO donor, glyceryl trinitrate (GTN).

Subjects and methods

Male Sprague-Dawley rats were housed and cared in accordance with the NH and MRC guidelines for animal experimentation with free access to tap water and standard chow (GR2 + pellets, 0.4% sodium chloride, 20% protein, Clarke King+Co, South Melbourne, Victoria, Australia), as previously described [16]. Diabetes was induced by an intravenous injection of streptozotocin (Sigma, St Louis, Missouri, USA) at a dose of 50 mg/kg body weight in citrate buffer, pH 4.5, at 8 weeks of age. Blood glucose levels were performed 3 days after streptozotocin injection and rats with blood glucose levels less than 15 mmol/l were excluded from the study. Ultratard Insulin (Novo-Nordisk A/C, Bagsvaerd, Denmark) 4 U/day was started 3 days after induction of diabetes to promote animal well-being without achieving euglycaemia. Age-matched rats from the same breed, injected with citrate buffer alone, served as controls.

Surgical preparation

The animals were prepared for renal function studies 3 weeks after induction of diabetes, as previously described [3]. Briefly, diabetic and control animals were anesthetized with an intraperitoneal injection of 40 mg/body weight methohexitone (Brietal, Eli Lilly, Indianapolis, Indiana, USA). Polyethylene catheters filled with normal saline were inserted into the left jugular vein (PE-45, internal diameter 0.58 mm, external diameter 1.52 mm with an external diameter 0.96) and the left carotid artery (PE-31, internal diameter 0.86 mm, external diameter 1.52 mm with an external diameter 0.96). Following vessel cannulations, a PE-65 polyethylene tubing (Brietal, Eli Lilly, Indianapolis, Indiana, USA) in 200 ul normal saline, was instituted via a jugular vein catheter (4 uCi/h/kg body weight; Amersham, Bucks, UK), diluted in normal saline, was instilled via a jugular vein catheter at a rate of 1 ml/h. After 120 min of equilibration, 20-30 min basal clearance periods were performed. The systolic blood pressure (SBP) was monitored continuously via an arterial catheter attached to a pressure transducer and blood pressure monitor (Hewlett-Packard 78205 A, Waltham, MA, USA) with measurements performed at 5-min intervals with the average value being calculated for each period. Urine was collected throughout the periods via a bladder catheter connected to preweighed 5 ml plastic tubes, and the volume was measured gravimetrically. Blood samples (300 µl) were taken from the arterial catheter at the midpoint of each period and blood loss replaced with an equivalent volume of normal saline.

In some animals the experiment was extended to evaluate acute effects of NO inhibition or NO donation. After completion of basal measurements, subgroups of control (n = 7) and diabetic (n = 8) rats were injected with an intravenous bolus of L-NAME (1 mg/kg body weight; Sigma, St Louis, Missouri, USA) in 200 ul normal saline. This dose was chosen based on studies which show that 1 mg/kg does not elicit a pressor response in diabetic animals [3]. The non-pressor dose of L-NAME has been deliberately chosen to avoid possible confounding influences of pressure natriuresis caused by higher doses of the substance [17,18]. Following 15 min of equilibration period another 30-min study clearance period was evaluated in a similar manner as described above.

To assess the renal response to NO donation, additional subgroups of control (n = 7) and diabetic (n = 8) rats were infused with glyceryl trinitrate (GTN). After completion of basal measurements the GTN was administered in a continuous infusion at a rate of 10 µg/min/kg body weight and a 30-min study clearance period was evaluated in a similar manner as described above after 15 min of equilibration. Finally, the animals were killed with an intravenous bolus of pentobarbitone sodium (Nembutal, Boehringer Ingelheim, NSW, Australia) and the kidneys were decapitated, removed and weighed.

Analytical methods

Plasma and urine 3H-inulin concentrations were determined by beta-counting of 10 µl aliquots for 5 min (Beckman, LS 3801, CA, USA) and concentrations of 125I-hippurate were determined in 75 µl samples counted for 3 min by gamma counter (Cobra II, Auto-Gamma 5000, Packard Instruments Company, Meriden, CT, USA). Beta activity of 125I was subtracted from 3H counts. Clearances of 3H-inulin, as a measure of glomerular filtration rate (GFR), and 125I-hippurate were calculated using the previously described formulas. Renal plasma flow (RPF) was determined as the quotient of hippurate clearance and the extraction factor for hippurate (0.81 for control and 0.70 for diabetic rats), as previously described [3]. Fractional filtration (FF) was calculated as the quotient of GFR and RPF. Plasma and urinary sodium and plasma glucose concentrations were measured on an autoanalyzer (Beckman, Palo Alto, CA, USA). Other values were calculated as follows: filtered load of sodium (FlNa) = PNa x GFR; fractional sodium reabsorption (FRNa) = 100 - [(UNa x V/PNa x GFR) x 100]; total sodium reabsorption (TRNa) = (PNa x GFR) - UNa x V, where PNa is plasma sodium concentration, UNa is urinary sodium concentration and V means urinary volume.
As a measure of renal NO production, the urine samples were assayed for NO\textsubscript{x} and cGMP production. Urinary NO\textsubscript{X} was measured as previously described [15] using the Griess reagent. Plasma samples were obtained from a subgroup of control and diabetic rats and assayed undiluted for measurement of plasma NO\textsubscript{X} levels. In brief, samples were incubated with nitrate reductase in the presence of NADPH to reduce all nitrate to nitrite (Boehringer Mannheim GMBH, Mannheim, Germany). After the enzyme incubation, the total NO\textsubscript{X} in the samples (representing both NO\textsubscript{X} and the reduced NO\textsubscript{X}) was measured using the Griess reagent and measured at 540 nm using a plate reader. Known concentrations of NaNo\textsubscript{X} were used to create a standard curve. Urinary cGMP was measured by radioimmunoassay (Amersham, Bucks, UK).

Statistical analysis

Data are expressed as means±SEM. GFR, RPF, urinary flow, U\textsubscript{Na} x V, TR\textsubscript{Na}, and urinary excretions of NO\textsubscript{X} and cGMP were expressed per kg body weight. All analyses were performed by analysis of variance, with repeated measures using Statview SE and graphics (Brainpower, Calabasas, CA, USA) on a Macintosh llsi Computer. A \( P \) value of less than 0.05 was viewed as statistically significant. Urinary cGMP was not normally distributed and therefore data for this parameter were analysed after logarithmic transformation. Data for this parameter are shown as median and 25th to 75th percentiles. Relationships of various variables were tested using multiple regression analysis with \( F \)-test value > 4. All tests were performed by analysis of variance, with repeated measures.

Results

The physical and laboratory parameters of the various groups of rats are summarized in Table 1. Diabetic rats exhibited hyperglycaemia and reduced weight gain. Haematocrit and kidney weight were not different between control and diabetic rats. However, the diabetic rats demonstrated an increase in kidney/body weight ratio. Renal functional parameters of control and diabetic rats are summarized in Table 2. There was a trend, although not statistically significant, for lower systolic blood pressures in diabetic rats. Basal GFR was markedly elevated in diabetic animals (3.7±0.3 ml/min) compared to control rats (2.0±0.1 ml/min). Diabetic rats also demonstrated a decrease in fractional sodium reabsorption. TR Na\textsubscript{X} was elevated in diabetic rats. There were no differences in plasma sodium concentration between control and diabetic rats.

To assess the contribution of various variables to the changes in renal sodium handling observed in the diabetic rats, renal sodium handling parameters were correlated with GFR, RPF, FF, SBP, blood glucose level, kidney weight, and urinary excretion of NO\textsubscript{X} and cGMP. In diabetic rats, multiple regression analysis revealed that there is a strong relationship between urinary sodium excretion and urinary excretion of NO\textsubscript{X} (\( P = 0.002 \)) and GFR (\( P = 0.008 \); Fig. 1). Fractional sodium reabsorption correlated negatively with urinary NO\textsubscript{X} (\( P = 0.004 \)). In contrast, there was a strong post-

Table 1. Physical and biochemical characteristics of the control and diabetic rats

<table>
<thead>
<tr>
<th></th>
<th>Control (( n = 25 ))</th>
<th>Diabetes (( n = 29 ))</th>
<th>Control + L-NAME (( n = 7 ))</th>
<th>Diabetes + L-NAME (( n = 8 ))</th>
<th>Control + GTN (( n = 7 ))</th>
<th>Diabetes + GTN (( n = 8 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>308±4.7</td>
<td>272±7*</td>
<td>308±14</td>
<td>281±10*</td>
<td>373±9</td>
<td>276±17*</td>
</tr>
<tr>
<td>Kidney weight (g)</td>
<td>3.15±0.1</td>
<td>3.0±0.1</td>
<td>3.0±0.1</td>
<td>3.1±0.2</td>
<td>2.9±0.1</td>
<td>3.1±0.1</td>
</tr>
<tr>
<td>Kidney/body weight ratio (%)</td>
<td>0.77±0.02</td>
<td>1.10±0.03*</td>
<td>0.75±0.03</td>
<td>1.11±0.05*</td>
<td>0.79±0.03</td>
<td>1.10±0.05*</td>
</tr>
<tr>
<td>Haematocrit</td>
<td>0.43±0.01</td>
<td>0.43±0.01</td>
<td>0.44±0.01</td>
<td>0.45±0.01</td>
<td>0.42±0.01</td>
<td>0.42±0.01</td>
</tr>
<tr>
<td>Blood glucose (mmol/1)</td>
<td>7.6±0.4</td>
<td>26.9±1.3*</td>
<td>7.3±0.9</td>
<td>25.0±2.0*</td>
<td>8.4±0.4</td>
<td>25.9±2.9*</td>
</tr>
</tbody>
</table>

Data are shown as means±SEM. *\( P < 0.001 \) diabetes vs controls.
The relationship between absolute sodium reabsorption and GFR was significant in diabetic rats ($P=0.0001$). No other correlations in diabetic rats were found between urinary sodium handling parameters and the variables listed above. In contrast, no such correlations were observed in control rats except for a relationship between total sodium reabsorption and GFR ($P=0.0001$). Stepwise regression analysis performed at the 95% probability level showed that the strongest relationship of $U_{\text{Na}}V$ to GFR (Step 1, $r=0.639$, $F=17.241$) with a weaker correlation obtained to urinary excretion of NO* (Step 2, $r=0.741$, $F=14.6$). Similar to the results obtained with multiple regression analysis, $FR_{\text{Na}}$, by stepwise regression, correlated only with urinary excretion of NO* (Step 1, $r=0.524$, $F=9.477$).

As summarized in Table 3, basal differences in renal haemodynamics and sodium handling in subgroups of control and diabetic rats treated with L-NAME were similar to those described previously. Diabetic rats demonstrated marked hyperfiltration and increases in RPF and FF associated with elevated urinary excretion of sodium, NO*, and cGMP compared to controls. There was a tendency for plasma NO* levels to be increased in the diabetic rats (control, $35\pm4$ vs diabetic, $45\pm6$ µmol/l, $P=0.10$). SBP was not different between both groups. Administration of L-NAME resulted in no change in SBP in diabetic rats, although there was a modest increase in SBP in the control animals ($P<0.05$ vs basal). In control rats there was no significant change in GFR after L-NAME whereas this intervention induced a significant decrease in GFR in diabetic rats. There was a significant decrease in RPF in both control and diabetic rats. However, the FF increased only in control rats. Administration of L-NAME had no effect on urinary flow and plasma sodium levels in control and diabetic rats. In contrast, NO inhibition was associated with a decrease in $U_{\text{Na}}V$ in both groups, although the difference was only statistically significant in diabetic rats ($P<0.05$). In control rats, this trend was apparent despite a slight increase in SBP after L-NAME. NO inhibition resulted in no change in $FR_{\text{Na}}$ in both groups of rats. However, there was a marked reduction in $TR_{\text{Na}}$ in the diabetic rats. Diabetic rats also demonstrated a significant decrease in $FL_{\text{Na}}$. No such change was observed in control animals. The changes in renal haemodynamics and sodium handling after L-NAME were accompanied by a significant reduction in the urinary NO* excretion but no change in urinary cGMP.

Data summarized in Table 4 show that basal differences in renal haemodynamics and sodium handling in control and diabetic rats were also apparent in animals treated with GTN. Diabetic animals demonstrated marked hyperfiltration and increases in RPF associated with elevated urinary excretion of sodium, NO*, and cGMP compared to control rats. Basal SBP was not different between both groups. A continuous infusion of GTN induced a mild decrease in SBP in both control and diabetic rats. In control rats, administration of GTN resulted in an increase in RPF and there was also a trend towards an increase in the GFR in the control group. These changes were associated
with significant increases in \textit{U}_\text{Na}V and urinary excretion of NO\textsubscript{3}. The modest changes in \textit{F}_\text{L}-\text{Na}, \textit{FR}_\text{Na}, \textit{TR}_\text{Na} and urinary excretion of cGMP observed in control animals did not reach statistical significance. In contrast to control animals, no significant renal responses to the GTN were observed in the diabetic animals. The relationship between GFR and \textit{U}_\text{Na}V, although not statistically significant, was still apparent after administration of L-NAME or GTN (Fig. 1, panels C and D).

**Discussion**

Diabetic hyperfiltering rats in the present study demonstrated increases in natriuresis and \textit{TR}_\text{Na} and a decrease in \textit{FR}_\text{Na}. These observations correspond to previous experimental reports \cite{4, 5}. The increase in \textit{U}_\text{Na}V has also been reported in hyperfiltering type 1 diabetic patients \cite{6, 7}. As in the present report, the increase in natriuresis has been shown in other studies to be associated with enhanced tubular sodium reabsorption. This phenomenon could at least in part be attributed to the activation of glucose–sodium co-transport \cite{6, 19}. Another mechanism which could explain increased sodium reabsorption in diabetes relates to the maintenance of glomerular–tubular balance when the filtered load of sodium rises. Marked elevation of the filtered sodium load was also observed in the diabetic animals in the present study.

**Table 3. Effect of L-NAME on systolic blood pressure, renal haemodynamics, sodium handling and urinary excretion of NO\textsubscript{3} and cGMP in control and diabetic rats**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>L-NAME</th>
<th>Diabetic</th>
<th>L-NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SBP (mmHg)</strong></td>
<td>133 ± 6</td>
<td>143 ± 9*</td>
<td>129 ± 5</td>
<td>129 ± 6</td>
</tr>
<tr>
<td><strong>GFR (ml/min/kg BW)</strong></td>
<td>4.9 ± 0.6</td>
<td>4.1 ± 0.9</td>
<td>13.3 ± 0.9*</td>
<td>10.3 ± 1.3*</td>
</tr>
<tr>
<td><strong>RPF (ml/min/kg BW)</strong></td>
<td>26.2 ± 2.7</td>
<td>15.3 ± 2.4*</td>
<td>47.5 ± 3.8</td>
<td>35.5 ± 5.3*</td>
</tr>
<tr>
<td><strong>FF</strong></td>
<td>0.19 ± 0.01</td>
<td>0.27 ± 0.02*</td>
<td>0.28 ± 0.01*</td>
<td>0.30 ± 0.02</td>
</tr>
<tr>
<td><strong>P\textsubscript{Na} (mmol/l)</strong></td>
<td>130 ± 2</td>
<td>150 ± 2</td>
<td>148 ± 3</td>
<td>150 ± 2</td>
</tr>
<tr>
<td><strong>\textit{U}_\text{Na}V (\textmu}mol/min/kg BW)</strong></td>
<td>5.5 ± 1.2</td>
<td>4.5 ± 1.0</td>
<td>28.1 ± 5.5*</td>
<td>211 ± 4.4*</td>
</tr>
<tr>
<td><strong>UF (\textmu}l/min/kg BW)</strong></td>
<td>71 ± 15</td>
<td>44 ± 7</td>
<td>287 ± 77*</td>
<td>228 ± 52*</td>
</tr>
<tr>
<td><strong>\textit{F}_\text{L}-\text{Na} (\textmu}mol/min/kg BW)</strong></td>
<td>0.73 ± 0.09</td>
<td>0.62 ± 0.13</td>
<td>1.96 ± 0.12*</td>
<td>1.52 ± 0.19*</td>
</tr>
<tr>
<td><strong>\textit{FR}_\text{Na} (%)</strong></td>
<td>0.73 ± 0.09</td>
<td>0.62 ± 0.13</td>
<td>1.91 ± 0.12*</td>
<td>1.50 ± 0.19*</td>
</tr>
<tr>
<td><strong>\textit{TR}_\text{Na} (\textmu}mol/min/kg BW)</strong></td>
<td>0.72 ± 2</td>
<td>0.62 ± 0.13</td>
<td>1.2 (1.1-5.4)*</td>
<td>3.3 (1.0-49.0)*</td>
</tr>
<tr>
<td><strong>\textit{UR}_\text{NO} (nmol/min/kg BW)</strong></td>
<td>0.8 (0.4-1.1)</td>
<td>0.5 (0.2-0.7)</td>
<td>12 ± 2</td>
<td>8 ± 1*</td>
</tr>
<tr>
<td><strong>\textit{Urinary cGMP} (pmol/min/kg BW)</strong></td>
<td>12 ± 2</td>
<td>8 ± 1*</td>
<td>71 ± 35*</td>
<td>30 ± 11*</td>
</tr>
</tbody>
</table>

Means ± SEM shown except for cGMP, where medians and 25th to 75th percentiles are shown. Abbreviations as in Table 2.

*P<0.05 vs control animals in the same period; †P<0.01 vs control animals in the same period; ‡P<0.05 vs basal; ¶P<0.01 vs basal.

**Table 4. Effect of GTN on systolic blood pressure, renal haemodynamics, sodium handling and urinary excretion of NO\textsubscript{3} and cGMP in control and diabetic rats**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>GTN</th>
<th>Diabetic</th>
<th>GTN</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SBP (mmHg)</strong></td>
<td>134 ± 6</td>
<td>130 ± 5</td>
<td>122 ± 5</td>
<td>117 ± 4</td>
</tr>
<tr>
<td><strong>GFR (ml/min/kg BW)</strong></td>
<td>6.2 ± 0.7</td>
<td>8.5 ± 0.9</td>
<td>12.1 ± 0.5*</td>
<td>12.1 ± 1.0*</td>
</tr>
<tr>
<td><strong>RPF (ml/min/kg BW)</strong></td>
<td>26.2 ± 1.8</td>
<td>40.4 ± 6.7†</td>
<td>51.7 ± 6.3†</td>
<td>51.7 ± 6.3†</td>
</tr>
<tr>
<td><strong>FF</strong></td>
<td>0.24 ± 0.02</td>
<td>0.23 ± 0.03</td>
<td>0.23 ± 0.02</td>
<td>0.23 ± 0.02</td>
</tr>
<tr>
<td><strong>P\textsubscript{Na} (mmol/l)</strong></td>
<td>156 ± 2</td>
<td>154 ± 1</td>
<td>154 ± 2</td>
<td>154 ± 2</td>
</tr>
<tr>
<td><strong>\textit{U}_\text{Na}V (\textmu}mol/min/kg BW)</strong></td>
<td>4.9 ± 2.0</td>
<td>7.4 ± 2.8†</td>
<td>20.4 ± 4.2†</td>
<td>21.4 ± 3.0†</td>
</tr>
<tr>
<td><strong>UF (\textmu}l/min/kg BW)</strong></td>
<td>62 ± 18</td>
<td>81 ± 20</td>
<td>214 ± 26†</td>
<td>219 ± 29†</td>
</tr>
<tr>
<td><strong>\textit{F}_\text{L}-\text{Na} (\textmu}mol/min/kg BW)</strong></td>
<td>1.0 ± 0.11</td>
<td>1.34 ± 0.12</td>
<td>1.89 ± 0.09†</td>
<td>1.81 ± 0.17</td>
</tr>
<tr>
<td><strong>\textit{FR}_\text{Na} (%)</strong></td>
<td>99.6 ± 0.1</td>
<td>99.4 ± 0.2</td>
<td>98.9 ± 0.2*</td>
<td>98.7 ± 0.3*</td>
</tr>
<tr>
<td><strong>\textit{TR}_\text{Na} (\textmu}mol/min/kg BW)</strong></td>
<td>0.98 ± 0.12</td>
<td>1.33 ± 0.14</td>
<td>1.85 ± 0.08*</td>
<td>1.79 ± 0.17</td>
</tr>
<tr>
<td><strong>\textit{UR}_\text{NO} (nmol/min/kg BW)</strong></td>
<td>6 ± 1</td>
<td>12 ± 4*</td>
<td>36 ± 10*</td>
<td>24 ± 6</td>
</tr>
<tr>
<td><strong>\textit{Urinary cGMP} (pmol/min/kg BW)</strong></td>
<td>1.9 (1.4-2.3)</td>
<td>2.0 (1.1-2.6)</td>
<td>4.6 (3.0-38.9)*</td>
<td>27 (2.9-92.9)*</td>
</tr>
</tbody>
</table>

Means ± SEM shown except for cGMP, where medians and 25th to 75th percentiles are shown. Abbreviations as in Table 2.

*P<0.05 vs control animals in the same period; †P<0.01 vs control animals in the same period; ‡P<0.05 vs basal; ¶P<0.01 vs basal.
due to glycosuria. In the experimental context, Bank et al. [4] reported that sodium restriction corrects the increased natriuresis and hyperfiltration in diabetic rats despite no effect on urinary volume or glucose excretion. Furthermore, the authors showed that the severely hyperglycaemic non-insulin-treated rats are able to preserve sufficient amounts of sodium during the osmotic diuresis. Since insulin is a potent antinatriuretic factor which acts predominantly at the distal nephron [20], one cannot exclude the effect of insulin treatment on renal tubular sodium handling in these diabetic rats. However, these animals had increased natriuresis which could partly relate to the model of streptozotocin diabetes being a state of insulinopenia. It must also be appreciated that diabetic rats have increased food intake [21] which is an important determinant of urinary sodium excretion.

There was a tendency for plasma NOX to be increased in the diabetic rats. However, the level of urinary NOX in these diabetic animals was at least 5-fold higher than in the control animals, these changes being much greater than the changes observed with respect to plasma NOX. Therefore, the present study confirms the previous report [1] that the increase in urinary NOX in experimental diabetes cannot be explained by an increase in the filtered plasma NOX.

The role of NO in the pathogenesis of renal haemodynamic changes in diabetes has been recently investigated [1–3]. It is possible that NO also contributes to altered renal sodium handling in diabetes. Although the processes involved in the natriuretic actions of NO remain to be fully established, it has been proposed that NO induces natriuresis by various mechanisms. Firstly, studies by Mattson and co-workers [12] have shown that natriuretic actions of NO are mediated by its ability to increase medullary blood flow. Secondly, NO exerts its physiological actions by stimulating its second messenger, cGMP [22], which has been shown to influence transport mechanisms in the cortical collecting duct cells in vitro [23,24]. Thirdly, in studies by Alberola et al. [11], an increase in fractional excretion of lithium (FELi) in volume-expanded dogs was significantly reduced by intrarenal administration of L-NAME. Since FELi is a marker of proximal sodium transport, these data suggest that proximal tubule may be one of the sites of the natriuretic actions of NO. Finally, NO may modulate renal excretory functions by antagonizing the actions of other substances such as angiotensin II, which are involved in the cortical collecting duct in diabetes [25,26].

Indeed, in diabetic rats, UNaV strongly correlated with urinary NOX and there was also a significant negative correlation of FRNa with urinary NOX excretion. However, diabetic rats also demonstrated a positive relationship between UNaV and GFR suggesting that increased natriuresis may in these animals be linked to increased filtered load of sodium rather than to increased renal activity of NO. This view is supported by the other findings of a marked increase in TRNa in diabetic rats and a strong association between this parameter and GFR. Therefore, in diabetic rats tubuloglomerular balance is preserved and diabetes-associated alterations in renal sodium handling are related to changes in renal haemodynamics rather than to the direct natriuretic actions of NO.

Further support for the view that a major influence of tubular sodium handling in diabetes is related to altered renal haemodynamics can be inferred from the changes following NO synthase inhibition. In non-diabetic rats NO inhibition with a non-pressor dose of L-NAME reduced UNaV in association with increases in tubular reabsorption. In contrast, in diabetic rats, although a decrease in UNaV after L-NAME was observed, FRNa remained unchanged and TRNa decreased markedly. Concomitant with these changes, L-NAME dramatically decreased GFR and RPF in the diabetic rats with a resultant marked reduction in the filtered load of sodium. Therefore, the response of renal sodium handling to L-NAME in the diabetic rats is most likely related to L-NAME-induced changes in renal haemodynamics rather than via direct actions by this NO synthase inhibitor on tubular sodium reabsorption.

Apart from NO, other humoral factors, such as atrial natriuretic factor (ANF), have been identified as mediators of diabetic hyperfiltration [27,28]. Therefore, in diabetes which is associated with elevated ANF levels which could presumably lead to increased natriuresis, the finding of increased sodium reabsorption along the nephron in diabetes is rather surprising. This suggests that mechanisms such as activation of glucose–sodium co-transport or maintenance of glomerular–tubular balance are major determinants of tubular sodium handling in moderately hyperglycaemic diabetic rats and counteract the actions of natriuretic substances such as ANF. It has been well established in clinical and experimental conditions that diabetes is a state of increased exchangeable sodium [21,29,30]. However, another possibility is that increased activities of natriuretic factors such as NO or ANF are secondary to the increased tubular sodium reabsorption with a net increase in exchangeable sodium, and are important factors in the maintenance of sodium homeostasis in the diabetic rat. It is possible that diabetic hyperfiltration is a result of these secondary changes such as increased ANF, a hormone which has been shown to increase GFR [27]. The role of ANF in diabetes has recently been further evaluated using ANF antagonists [28,31]. These studies have confirmed a role for this peptide in diabetic hyperfiltration. Furthermore, recent studies have indicated that ANF antagonism not only decreases urinary cGMP in diabetic rats but also is associated with a decrease in natriuresis in these animals [31,32]. These findings suggest that changes in urinary cGMP excretion in diabetes are related more to the actions of ANF than to NO and cannot be used as a marker of NO synthase activity in this model.

The lack of evidence in the present study that hyperproduction of NO in the diabetic kidney is involved in the tubular sodium handling alterations in diabetes further extends our previous findings of blunted pressure natriuresis in experimental diabetes.
[3]. Since NO [33,34] and ANF [35] have been implicated in mediating pressure natriuresis, it is possible that diabetic tubular cells do not respond adequately to these factors or to cGMP which is the second messenger for both NO and ANF. Several groups have reported a resistance to the natriuretic action of ANF in IDDM patients with sodium retention [36,37].

To further explore the role of NO, the NO-donating agent glyceryl trinitrate was administered to control and diabetic rats. Although the effect of GTN on systemic blood pressure was similar in control and diabetic animals, control rats responded to NO donation with an increase in RPF, increased natriuresis and a rise in urinary excretion of NOX. The fact that no such responses to the administration of the NO donor were observed in the diabetic rats is in agreement with the above stated hypothesis of decreased renal responsiveness to natriuretic factors in diabetes. The post-GTN renal haemodynamic pattern in the control animals was similar to that observed in diabetic animals during the basal periods. Therefore, in summary, the changes in renal sodium handling in diabetes which occur in response to NO blockade appear to relate to the effects on renal haemodynamics rather than to direct actions of NO on tubular cells.

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NO and renal sodium handling in diabetes


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