Chronic renal denervation prevents glomerular hyperfiltration in diabetic rats

Gerd Luippold, Martina Beilharz and Bernd Mühlbauer

Department of Pharmacology and Toxicology, Faculty of Medicine, University of Tübingen, Tübingen, Germany

Abstract

Background. The increase in glomerular filtration rate (GFR) induced by amino acid infusion is attenuated in rats with chronic renal denervation. The aim of the present study was to investigate whether renal denervation abrogates glomerular hyperfiltration occurring in the early state of diabetes mellitus.

Methods. Sprague–Dawley rats were subjected to bilateral renal denervation before induction of diabetes mellitus (DM) by streptozotocin. Clearance experiments were performed 2 weeks after onset of moderate DM. Glomerular volume was estimated following paraformaldehyde fixation in rat kidney slices from measurement of cross-sectional area of Bowman’s capsule.

Results. GFR in non-diabetic rats with intact nerves (CON-INN) was $0.82 \pm 0.03$ ml$^{-1}$min$^{-1}$100 g$^{-1}$ body weight. Diabetic animals with innervated kidneys presented a significant glomerular hyperfiltration ($1.13 \pm 0.03$ ml$^{-1}$min$^{-1}$100 g$^{-1}$ body weight), while renal denervation in diabetic rats lowered GFR towards levels of CON-INN ($0.88 \pm 0.03$ ml$^{-1}$min$^{-1}$100 g$^{-1}$ body weight). Estimated glomerular volume amounted to $0.69 \pm 0.03 \times 10^6 \mu m^3$ in the CON-INN group and was significantly higher in diabetic animals with intact renal nerves ($0.86 \pm 0.04 \times 10^6 \mu m^3$). Interestingly, renal denervation prevented the glomerular enlargement due to DM.

Conclusions. Renal nerves appear to be significantly involved in the mediation of glomerular hyperfiltration in experimental DM. If the kidney is prevented from sympathetic nerve stimulation, structural changes due to early diabetic nephropathy, i.e. glomerular enlargement, are abolished.

Keywords: diabetes mellitus; glomerular hyperfiltration; rats; renal denervation

Introduction

In 1934, Cambier suggested that the glomerular filtration rate (GFR) is elevated in patients with diabetes mellitus [1]. Indeed, between 25 and 40% of patients with insulin-dependent diabetes mellitus (IDDM) have a GFR above the normal range of age-matched healthy non-diabetic subjects. Glomerular hyperfiltration is especially pronounced in patients with newly diagnosed IDDM and during intervals of poor metabolic control. In longitudinal studies it was proposed that hyperfiltration serves as a risk factor for subsequent albuminuria and development of diabetic nephropathy in IDDM patients [2]. As reviewed by Christiansen, metabolic residuals, vasoactive hormones, the autonomic nervous system and increased kidney and glomerulus size have been proposed as mediators of the GFR elevation in diabetes [3]. However, to date the responsible mediators as well as underlying mechanisms involved in the diabetes-induced renal haemodynamic abnormalities are incompletely understood.

Corresponding to the glomerular hyperfiltration in the early state of diabetes mellitus, an increase in GFR also occurs during ingestion of proteins [4] or i.v. infusion of amino acids [5]. The glomerular hyperfiltration induced by amino acids is attenuated in rats which underwent chronic renal denervation, suggesting that the renal nerves are essential in the response to systemic amino acid load [6]. The aim of the present study was to evaluate whether renal nerves also control the increase in GFR associated with the development of diabetes mellitus.

For this purpose, clearance experiments were performed in rats with streptozotocin-induced diabetes mellitus. Before onset of diabetes, rats were subjected to...
bilateral renal denervation. In addition, glomerular volume was estimated following paraformaldehyde fixation in rat kidney slices from measurement of cross-sectional area of Bowman’s capsule.

**Subjects and methods**

**Animals and renal denervation**

All animal experimentation was conducted in accordance with the German Law on the Protection of Animals. Before induction of diabetes mellitus, male Sprague-Dawley rats (Charles River, Sulzfeld, Germany) were subjected to bilateral renal denervation or sham operation. The rats had an initial body weight of 200–225 g. Renal denervation (DNX) was performed as described previously [6]. In brief, rats were anaesthetized with a mixed solution of ketamine (Parke-Davis, Freiburg, Germany) and xylazine (Bayer, Leverkusen, Germany) in isotonic saline. Following abdominal midline incision both kidneys were exposed and the renal arteries and veins were isolated from connective tissue. After stripping the visible nerves, the vessels were painted for 2 min with a solution of 10% phenol in absolute ethanol. The muscular layer of the abdominal wall was sutured with resorbive material (Vicryl 2-0, Ethicon, Hamburg, Germany) and the skin was closed by non-resorbive filament (Dermafil 2-0, Dr Ruhland, Neustadt, Germany). Animals recovered from anaesthesia 10–20 min after the end of surgery. In sham operated animals the renal nerves were isolated but preserved. As described earlier [6], correct denervation was assessed by measuring the renal tissue content of catecholamines using a HPLC method. Completeness of denervation was assumed if the norepinephrine tissue content was <10% of the mean value in the sham operated groups.

**Induction of diabetes mellitus**

Diabetes mellitus was induced 3 days following renal denervation or sham operation by an i.p. injection of 60 mg kg\(^{-1}\) body weight streptozotocin (STZ; Sigma Chemicals, Deisenhofen, Germany) dissolved in sodium citrate buffer (pH 4.0). Rats were included in the diabetic groups if blood glucose levels, which were measured 24 h after STZ injection in capillary tail blood samples, ranged between 250 and 350 mg dl\(^{-1}\). In using this protocol, insulin substitution was not required throughout the study. Non-diabetic rats receiving STZ-vehicle served as controls. The animals were allowed free access to a regular rat pellet diet (Altromin 1320\(^{®}\), Altromin, Lage, Germany). Experiments were performed in four different groups: Group 1, rats which underwent bilateral renal denervation before induction of diabetes mellitus (DNX; \(n = 10\)). Group 2, rats which underwent sham operation before induction of diabetes mellitus (DM-INN; \(n = 9\)). Group 3, non-diabetic rats which underwent bilateral renal denervation (CON-DNX; \(n = 6\)). Group 4, sham operated non-diabetic rats with intact kidneys (CON-INN; \(n = 9\)).

**Metabolic cage experiments**

Metabolic cage experiments were performed 10–12 days following onset of diabetes mellitus. Therefore, rats were placed in metabolic cages (Tecniplast, Hohenpeissenberg, Germany) for 24 h to measure food and fluid intake, blood glucose, urinary volume and sodium excretion.

**Measurement of renal haemodynamics**

Two weeks after onset of diabetes mellitus, rats were anaesthetized with an i.p. injection of thiopental sodium (80 mg kg\(^{-1}\); TRAPANAL\(^{®}\), Byk Gulden, Konstanz, Germany) and placed on a heated table (RT\(^{®}\), Effenberger, München, Germany), which was thermo-controlled to keep the rectal temperature constant at 37.2°C. Following tracheostomy, two polyethylene (PE)-catheters were inserted into the right jugular vein for i.v. infusion. Another PE-catheter was placed into the left carotid artery for blood sampling and continuously monitoring of arterial blood pressure by means of an electronic transducer (TBM 4\(^{®}\), WPI, Heidelberg, Germany) connected to a recorder (WK 280\(^{®}\), WKK, Kaltbrunn, Switzerland). After a small supra-pubic midline incision, a PE-catheter (dead space below 20 μl) was inserted into the bladder for urine collection. After surgical preparation Ringer solution (in millimolar concentrations: 111 NaCl, 30 NaHCO\(_3\), 4.7 KCl) was intravenously infused at a rate of 6.6 ml h\(^{-1}\) over 30 min, followed by a sustained infusion of 1 ml h\(^{-1}\)100 g\(^{-1}\) body weight. Via the second i.v. catheter \([^3H]\)inulin (1.2 μCi ml\(^{-1}\)) and \([^{14}C]\)para-aminohippuric acid (PAH; 1.0 μCi ml\(^{-1}\)) dissolved in Ringer were infused at a rate of 0.6 ml h\(^{-1}\) throughout the entire experiment.

The animals were allowed to recover from surgical procedure for 60 min before beginning of the clearance experiments. Two clearance periods of 20 min duration each were carried out during continuous infusion of Ringer solution. Plasma samples were drawn at the midpoint of each clearance period. After termination of the last clearance period, samples of peripheral arterial (\(P_A\)) and renal venous blood (\(P_{RV}\)) were drawn simultaneously to estimate renal PAH extraction (\(E_{PAH}\)) from the \(^{14}C\)PAH-activity according to the following formula:

\[
E_{PAH} = (P_A - P_{RV})/P_A
\]

GFR and renal plasma flow (RPF) were determined as the renal clearance of insulin and PAH, respectively. RPF was corrected for the PAH extraction. Filtration fraction was calculated according to the usual formula.

**Fixation of the kidney**

After clearance experiments had been completed, the left kidney was removed decapsulated and weighted. The right kidney was perfused via carotid artery. The aorta was subsequently ligated distally to the right renal artery and the kidney was perfused with 5% paraformaldehyde in phosphate buffer (pH 7.4).

**Analytical methods**

Urine volume and kidney wet weight were measured gravimetrically. Blood samples were centrifuged, and the haematocrit was assessed. Urinary and plasma concentrations of sodium were determined by flame photometry (ELEX 6361\(^{®}\), Eppendorf, Hamburg, Germany), the \([^{3}H]\)inulin and \([^{14}C]\)PAH radioactivities were measured by liquid phase scintillation counting (2550 TR\(^{®}\), Canberra Packard,
Frankfurt, Germany). Blood glucose levels were determined by a glucometer (REFLOLUX®, Boehringer Mannheim, Germany). Urinary dopamine concentrations were analysed using an HPLC method as described previously [6].

After perfusion the right kidney was cut into 2 mm thick slices using a razor blade. The slices were post-fixed in the perfusion medium, dehydrated and embedded in paraffin. From each rat one randomly sampled PAS-stained slice was used and studied by confocal microscopy (LSM 410, Zeiss, Jena, Germany). Per animal the cross-sectional area (A) of 30 representative superficial glomeruli was analysed. The individual radius (r) of the glomeruli was determined by $r = (A/\pi)^{1/2}$. The mean glomerular volume (V) was estimated by the following formula:

$$V = \left(\frac{4}{3}\pi r^3\right)$$

**Statistical analysis**

Both clearance periods were calculated individually and summarized as mean of groups (±SEM). Statistical analysis of the differences among groups was performed by the analysis of variance (ANOVA) with Bonferroni correction for multiple comparisons. P values <0.05 were considered to be significant.

**Results**

**Characteristics of experimental animals**

After 10–12 days following onset of diabetes mellitus induced by STZ, blood glucose was elevated and body weight gain was reduced when compared with non-diabetic controls (Table 1). These changes were similar in diabetic rats with intact kidneys or after renal denervation. As depicted in Table 1, metabolic cage experiments showed that in the diabetic groups fluid and food intake as well as urine output were markedly increased in comparison with non-diabetic controls. According to the higher food consumption and consequently sodium intake urinary sodium excretion was elevated in diabetic rats as well (Table 1).

**Effect of renal denervation on renal function**

In clearance experiments, urinary flow rate was not significantly altered in rats with diabetes mellitus when compared with non-diabetic controls (Table 2). Urinary sodium excretion showed a tendency towards elevated levels in diabetic rats; however, the effect did not reach the level of significance. Urinary excretion of dopamine was similar in rats with denervated and innervated kidneys. There was a slight, however statistically insignificant, increase in urinary dopamine excretion in diabetic rats (Table 2).

GFR was similar in non-diabetic rats with innervated and denervated kidneys. After onset of STZ-induced diabetes mellitus, a significant glomerular hyperfiltration with an increase by 38% was observed in rats with innervated kidneys when compared with non-diabetic controls (Figure 1). Renal denervation prevented the increase in GFR due to diabetes mellitus almost completely (Figure 1). RPF, as measured by renal clearance of PAH, was similar in non-diabetic rats with innervated and denervated kidneys (Figure 2).

---

**Table 1.** Metabolic cage experiments in non-diabetic (CON) and diabetic (DM) rats with intact renal nerves (INN) or after renal denervation (DNX)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Body weight (g)</th>
<th>Fluid intake (ml-24 h⁻¹)</th>
<th>Food intake (g-24 h⁻¹)</th>
<th>Blood glucose (mg-dl⁻¹)</th>
<th>UV (ml-24 h⁻¹)</th>
<th>UNaV (mmol-24 h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON-INN</td>
<td>9</td>
<td>310±7</td>
<td>38±2</td>
<td>32±2</td>
<td>89±9</td>
<td>15±1</td>
<td>2.02±0.30</td>
</tr>
<tr>
<td>CON-DNX</td>
<td>6</td>
<td>312±15</td>
<td>42±3</td>
<td>34±2</td>
<td>98±8</td>
<td>13±1</td>
<td>2.09±0.30</td>
</tr>
<tr>
<td>DM-INN</td>
<td>9</td>
<td>254±11</td>
<td>221±14</td>
<td>51±2</td>
<td>401±23</td>
<td>172±15</td>
<td>4.14±0.35</td>
</tr>
<tr>
<td>DM-DNX</td>
<td>10</td>
<td>247±8</td>
<td>210±13</td>
<td>44±3</td>
<td>430±25</td>
<td>183±8</td>
<td>3.15±0.37</td>
</tr>
</tbody>
</table>

Values represent mean (±SEM). UV, urinary flow rate; UNaV, urinary sodium excretion. *P<0.05, vs respective CON group.

**Table 2.** Clearance experiments in non-diabetic (CON) and diabetic (DM) rats with intact renal nerves (INN) or after renal denervation (DNX)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>MAP (mmHg)</th>
<th>HR (min⁻¹)</th>
<th>Hct (%)</th>
<th>UV (ml-min⁻¹.100 g⁻¹)</th>
<th>UNaV (µmol-min⁻¹.100 g⁻¹)</th>
<th>FENa (%)</th>
<th>UDAV (pmol-min⁻¹.100 g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON-INN</td>
<td>9</td>
<td>108±3</td>
<td>387±7</td>
<td>44±1</td>
<td>12.2±2.7</td>
<td>0.82±0.21</td>
<td>0.67±0.16</td>
<td>5.05±0.61</td>
</tr>
<tr>
<td>CON-DNX</td>
<td>6</td>
<td>109±4</td>
<td>388±5</td>
<td>47±1</td>
<td>14.2±4.4</td>
<td>1.18±0.23</td>
<td>0.97±0.19</td>
<td>6.43±0.70</td>
</tr>
<tr>
<td>DM-INN</td>
<td>9</td>
<td>106±2</td>
<td>318±11</td>
<td>45±1</td>
<td>13.3±3.1</td>
<td>1.39±0.43</td>
<td>0.93±0.32</td>
<td>7.31±0.55</td>
</tr>
<tr>
<td>DM-DNX</td>
<td>10</td>
<td>104±4</td>
<td>350±8</td>
<td>47±1</td>
<td>11.6±2.5</td>
<td>1.51±0.27</td>
<td>1.37±0.40</td>
<td>7.19±0.89</td>
</tr>
</tbody>
</table>

Values represent mean (±SEM). MAP, mean arterial blood flow; HR, heart rate; Hct, haematocrit; UV, urinary flow rate; UNaV, urinary sodium excretion; FENa, fractional excretion of sodium; UDAV, urinary dopamine excretion.

*aP<0.05 vs respective CON group.

*bP<0.05 vs DM-INN group.
mellitus, in contrast, induced a significant elevation in RPF in rats with intact renal innervation (Figure 2). This effect was abrogated in diabetic rats with denervated kidneys. Because of the almost parallel changes in GFR and RPF, the calculated filtration fraction ranged between 23±2 and 25±2% and did not significantly vary among the different groups. The results suggest that bilateral renal denervation is effective in preventing the diabetes-induced glomerular hyperfiltration and hyperperfusion.

Mean arterial blood pressure was similar among the groups (Table 2). However, a significantly lower heart rate was observed in diabetic rats, irrespective of renal nerve supply. Haematocrit did not vary in the different groups, even though it showed a tendency towards higher levels in rats with renal denervation (Table 2). Norepinephrine content in the denervated kidneys was significantly decreased by >90% (CON-DNX: 10.6±0.7 ng·g wet weight⁻¹; DM-DNX: 8.1±0.8 ng·g wet weight⁻¹) when compared with the sham-operated kidneys (CON-INN: 149.3±14.3 ng·g wet weight⁻¹; DM-INN: 165.1±18.7 ng·g wet weight⁻¹), indicating that the denervation procedure was correct.

Effect of renal denervation on glomerular and kidney hypertrophy

In diabetic rats, renal hypertrophy was observed after 2 weeks, as shown by an increase in the left kidney wet weight per body weight (0.36±0.01 to 0.49±0.02 g·100 g⁻¹ body weight). However, renal denervation did not attenuate the diabetes-induced increase in kidney wet weight (Figure 3).

Planimetric examination of kidney slices 2 weeks after onset of diabetes mellitus revealed a remarkable difference in calculated glomerular volume among the four groups. Diabetic rats with innervated kidneys exhibited a clear enlargement of the glomerular volume, which was significantly attenuated by renal denervation (Figure 4). Per se renal denervation did not alter glomerular volume, as demonstrated for non-diabetic
controls. Taken together, the data indicate that renal denervation suppresses the glomerular hypertrophy in diabetic rats, but not the increase in kidney wet weight.

Discussion

Results of the present study suggest that renal nerves are functionally involved in intrarenal haemodynamic abnormalities observed in the early state of experimental diabetes mellitus. In particular, chronic bilateral renal denervation normalizes the diabetes-induced glomerular hyperfiltration and attenuates the increase in renal plasma flow. Associated with these haemodynamic changes, renal denervation also prevents the glomerular enlargement induced by diabetes mellitus. However, renal denervation did not influence the diabetes-related kidney hypertrophy.

It is generally agreed that, under physiological conditions, basal renal nerve activity did not influence renal haemodynamics. For instance, in conscious dogs and humans, surgical or pharmacological renal denervation did not affect renal blood flow or renal vascular resistance [7,8]. These findings are in accordance with our results showing that GFR and RPF were similar when comparing non-diabetic rats after chronic renal denervation and sham operated controls. In the present study, onset of experimental diabetes mellitus significantly increased GFR and RPF in rats with intact kidneys, an effect which has been found by others within the first few weeks of diabetes [9]. These changes in renal haemodynamics were accompanied by enlargement of glomerular volume and kidney wet weight. In the present experiments, chronic renal denervation significantly attenuated the diabetes-induced increase in glomerular volume and kidney weight.

In the present experiments, chronic renal denervation significantly attenuated the diabetes-induced increase in glomerular volume and kidney weight. However, the diabetes-induced kidney hypertrophy was not affected by renal denervation. One reason for this observation is that glomerular enlargement in diabetic rats may occur without detectable changes in kidney weight because glomeruli account for only ~2% of total renal volume.

Some clinical and experimental data have suggested that glomerular hyperfiltration represents an initiating mechanism for diabetic nephropathy. For instance, Mogensen demonstrated that diabetic patients with high GFRs early in the disease had a greater risk of progression to diabetic nephropathy [10]. According to the data of the present study renal nerves play a significant role in the regulation of diabetes-induced glomerular hyperfiltration. In this respect, the involvement of renal nerves in the development of diabetic nephropathy has been studied by Matsuoka [11] in diabetic rats. He reported that albuminuria, representing a parameter of early diabetic nephropathy, was 3.5-fold higher in animals with renal denervation than in sham operated animals after 6 weeks of observation. These results indicate that renal denervation appears to aggravate the progress of diabetic nephropathy in STZ-treated rats as judged through assessment of urinary albumin excretion [11]. These conflicting results when compared with the findings of the present study, in which a beneficial effect of renal denervation was proposed, might be caused by various study designs, the evaluation of different study parameters (albuminuria vs glomerular hyperfiltration), the application of insulin or, even more important, the problem of renal reinnervation. Functional reinnervation has been reported to begin 3 weeks post-denervation with a complete return of function by 8 weeks [12]. Another study explored the effect of bilateral renal denervation on diabetic nephropathy in Otsuka Long-Evans Tokushima Fatty rats, a model for non-insulin dependent diabetes mellitus (NIDDM) [13]. In this study, urinary protein excretion was significantly lower in rats with denervated kidneys than in sham operated controls. The glomerular matrix score, a morphological indicator of early nephropathy, was reduced in rats 4 weeks after renal denervation, suggesting that the lack of sympathetic stimulation retards the progression of glomerulosclerosis in NIDDM rats [13]. Taken together, the role of renal nerves in the development of diabetic nephropathy is something puzzling and has to be further investigated.

The fact that denervation attenuates the abnormalities of renal haemodynamics induced by diabetes mellitus, as shown in the present study, may offer additional insights into the underlying mechanisms. The release of the main transmitter in the peripheral sympathetic nerve terminals, norepinephrine, is ultimately controlled by the firing rate of the renal nerves. However, the amount of norepinephrine released at the neuroeffector junction can be modulated by presynaptically located receptors [14]. For instance, presynaptic dopamine receptors of the D2-like family inhibit norepinephrine release in kidney slices [15]. Thus, activation of presynaptic D2-like receptors via endogenous dopamine might functionally inhibit the norepinephrine-induced renal vasoconstricting effects and consecutively increase GFR and renal blood flow.

With regard to a reduced renal vascular resistance, diabetes-induced glomerular hyperfiltration has been abolished by subchronic administration of the dopamine D2-like receptor antagonist domperidone [16], indicating that glomerular hyperfiltration is associated with a stimulated dopaminergic system. Under the assumption of an increased intrarenal synthesis and interstitial release of dopamine in the early state of diabetes mellitus, it might be speculated that the neuronal release of norepinephrine is reduced via activation of presynaptic D2-like receptors. Such reduction in presynaptic norepinephrine release may contribute, at least in part, to the development of glomerular hyperfiltration due to diabetes mellitus. Interruption of this mechanism by chronic renal denervation is therefore suspected to contribute to the normalization of GFR in diabetic rats with denervated kidneys.

An alternative explanation of the present results includes the existence of dopamine-containing renal nerves. In the dog, renal tissue dopamine concentration
is substantially reduced following renal denervation, suggesting that renal nerves are the major source of renal dopamine content [17]. Since renal sympathetic nerve activity is increased in diabetes mellitus the hypothesized neuronal release of dopamine might induce a vasodilatory effect on renal vasculature, inducing an increase in GFR in diabetic rats. In this respect renal denervation would prevent the release of dopamine and normalize the diabetes-induced glomerular hyperfiltration as observed in the present experiments. However, it may be argued that the small differences in the urinary dopamine excretion as shown in the present study do not support the notion of changes in neuronal dopamine release. Possibly, the urinary dopamine excretion is not a sensitive parameter for intrarenal changes of dopamine concentration.

In consideration of the effect of renal denervation on the vasculature of the kidney the issue of hypersensitivity has to be mentioned. Studies comparing the effect of catecholamines on renal haemodynamics in innervated and denervated kidneys outline that chronic denervated kidneys (7–10 days) exhibit supersensitivity to norepinephrine [18]. Conversely, other reports indicate that the denervated kidney does not become supersensitive to physiological levels of circulating norepinephrine [19]. Both pre- and postsynaptic mechanisms contribute to hypersensitivity [20]. The presynaptic mechanism is represented by the loss of norepinephrine uptake into the renal sympathetic nerve terminals. As a postsynaptic mechanism the up-regulation of the postsynaptic adrenoceptors has been proposed. Since we could not clarify in the present experiments whether an increased responsiveness of the renal vasculature to circulating catecholamines might contribute to the normalization of GFR in diabetic animals, this issue remains to be investigated in further experiments.

In conclusion, the present data show that renal nerves are significantly involved in the regulation of renal haemodynamics in experimental diabetes mellitus. The mechanism of the effect of renal denervation on restoration of normal GFR in diabetic animals remains to be determined.

Acknowledgements. We wish to thank Dr A. Mack and PD Dr M. Wehrmann for helpful support in the estimation of glomerular volume and Frau E. Hoffmann for excellent technical assistance. Financial support for this study was provided by the Federal Ministry of Education and Research (Grant No. 01EC9405), the Interdisciplinary Center of Clinical Research (IZKF) Tübingen (Grant No. 01KS9602) and the Deutsche Forschungsgemeinschaft (DFG-Grant Mu 1297/1-2).

Conflict of interest statement. None declared.

References


Accepted in revised form: 24.9.03
Received for publication: 20.12.02