Renal Sympathetic Nervous System Hyperactivity in Early Streptozotocin-Induced Diabetic Kidney Disease

Ibrahim M. Salman,1,4 Omar Z. Ameer,1 Munavvar A. Sattar,1 Nor A. Abdullah,2 Mun F. Yam,1,3 Ghassan Z. Abdullah,1 Muthanna F. Abdulkarim,4 Md. Abdul Hye Khan,4 and Edward J. Johns5

1Department of Cardiovascular and Renal Physiology and Pharmacology, School of Pharmaceutical Sciences, Universiti Sains Malaysia, Minden, Penang, Malaysia
2Faculty of Medicine, Department of Pharmacology, Universiti Malaya, Kuala Lumpur, Malaysia
3Faculty of Medicine and Health Science, Department of Anatomy, Universiti Putra Malaysia, Serdang, Selangor, Malaysia
4Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, Wisconsin, USA
5Department of Physiology, University College Cork, Cork, Ireland

Aim: We assessed the role of renal sympathetic nervous system in the deterioration of renal hemodynamic and excretory functions in rats with streptozotocin (STZ)-induced diabetic kidney disease (DKD). Methods: Male Sprague–Dawley (SD) rats were induced with diabetes mellitus (DM) using STZ (55 mg/kg, i.p.). The acute studies were conducted on denervated anesthetized rats 7 days after STZ administration. Two sets of experiments were performed: clearance experiments in which six 20-min urine and plasma collections were carried out to measure kidney function parameters, and hemodynamic experiments in which the renal nerves were electrically stimulated and responses in renal vascular resistance (RVR) and renal blood flow (RBF) were recorded. Results: Renal denervation in STZ-induced diabetic rats produced higher fractional excretion of sodium (FENa) but lower plasma sodium (PNa), glomerular filtration rate (GFR), and plasma creatinine (PCr) (all P < 0.05 vs. innervated diabetic rats). In innervated diabetic rats, renal nerve stimulation (RNS) caused significant attenuation in the renal vasoconstrictor responses (all P < 0.05 vs. innervated control). Renal denervation in diabetic rats significantly blunted these responses (all P < 0.05 vs. innervated diabetic rats); however, they were significantly higher (all P < 0.05) while compared to denervated control counterparts. Conclusions: The data demonstrate an early role for the renal sympathetic innervation in the pathogenesis of DKD. If the kidney is prevented from renal sympathetic nerve action renal functional parameters are markedly improved. The data further suggest an early enhancement in renal sensitivity to intrarenal norepinephrine (NE) upon the removal of renal sympathetic tone in STZ-induced diabetic rats. Neurourol. Urodynam. 30:438–446, 2011. © 2011 Wiley-Liss, Inc.

Key words: diabetic kidney disease; renal denervation; renal nerve stimulation; renal sympathetic nerve; streptozotocin

INTRODUCTION

Diabetic kidney disease (DKD) develops in approximately one-third of all patients with diabetes. Among the major morbidity and mortality factors confronted by diabetic patients is an increased risk of developing diabetic nephropathy that often perpetuates to end-stage renal disease (ESRD).1–3 A long-standing question pertaining to the occurrence of DKD concerns the mechanisms involved in this pathological process. A wealth of data has been generated on possible mechanisms by which diabetes and its ancillary metabolic, hemodynamic, growth, and glomerular cell injury-related alterations may modulate the progression of diabetic nephropathy.4–7 Nevertheless, the observation that approximately two-thirds of patients with diabetes do not develop DKD suggests that hyperglycemia is a permissive factor in diabetic nephropathy, but elevated plasma glucose levels alone is not completely responsible for renal injury.4

The early stages of DKD induced by streptozotocin (STZ) are characterized by renal hemodynamic changes leading to a wide range of glomerular filtration rate (GFR) from normal to high values in humans8,9 and in animals.10,11 A recent investigation form our laboratory team has shown that early renal functional and hemodynamic responses to acute-stage type 1 diabetes mellitus (DM) by STZ exhibit a range of physiological and biochemical maladaptive changes mainly characterized by the likely existence of preenal acute renal dysfunction, such as glomerular hyperfunction, hypotonic urinary flow, and sustained elevations in plasma sodium (PNa) and plasma creatinine (PCr).12 Of particular interest is the hemodynamic phenotype in early diabetes which is characterized by glomerular hyperfiltration, a likely prerequisite for progressive diabetic nephropathy.9,13 Hyperfiltration does not rely upon accumulation of NaCl in the body, because GFR can increase relentlessly in early diabetes, notwithstanding a decline in extracellular volume.14 Glomerular hyperfiltration has been correlated with abnormalities of the glomerulus and preglomerular vessels, although specific mechanisms have not been fully delineated.10,15 It has been proposed that in recent onset DM there is increased proximal tubular reabsorption.16 Accordingly, more of the glomerular filtrate is reabsorbed and less reaches the macula densa at the end of Henle’s loop. This causes GFR to increase through the normal physiologic action of the tubuloglomerular feedback (TGF) system.17

© 2011 Wiley-Liss, Inc.
Several lines of evidence have suggested that an early state of imbalance between nitric oxide (NO) pathway and renin-angiotensin system (RAS) leading to an enhancement in the peripheral sympathetic nervous system activity is implicated in the pathogenesis of renal dysfunctions accompanying DM. Nonetheless, a considerable paucity of information still exists on some aspects of this issue. Particularly, a potential contribution of renal sympathetic nerve activity in the altered renal functional and hemodynamic responses observed in recent onset DKD has not been thoroughly investigated.

Christiansen proposed that metabolic residuals, vasoactive hormones, the autonomic nervous system and increased kidney and glomerular size are likely to be the responsible mediators for GFR elevation during intervals of poorly controlled insulin-dependent DM (type I DM). Luippold et al. further reported that chronic renal denervation prior to the induction of STZ-induced DM possessed a considerable renoprotective action against glomerular hyperfiltration. These findings support the view that adrenergic components are directly related to the impact on renal and hemodynamic functions in uncontrolled DM. However, to date the responsible mediators as well as underlying mechanisms involved in DKD are incompletely understood.

Against this backdrop, a hypothesis is formulated that under this pathophysiological condition sustained renal sympatho-excitation might play a contributory role in mediating the early functional and hemodynamic phenotypes of an STZ-induced diabetic kidney. In view of that, the current study addresses this issue by evaluating the role of renal sympathetic tone in the prototypical reductions in renal hemodynamic and excretory functions accompanying STZ-induced DM. This was carried out by employing clearance studies and evaluating the changes in renal blood flow (RBF) and renal vascular resistance (RVR) caused by direct electrical stimulation of renal nerves prior to and following acute renal denervation (ARD) in an STZ-induced rat model of DM.

**MATERIALS AND METHODS**

**Animals**

The experimental procedures described herein conformed with the guidelines and practices of the Animal Ethics Committee, Universiti Sains Malaysia (USM), Penang, Malaysia. Male Sprague–Dawley (SD) rats, weighing 280–350 g, were fed a standard pellet diet (Gold Coin Sdn Bhd, Malaysia) of the following composition: crude protein (min 21–23%), crude fiber (max 5%), crude fat (minimum 3%), moisture (maximum 13%), ash (maximum 8%), calcium (0.8–1.2%) and phosphorus (0.6–1%), and had free access to water. Rats were randomly allotted in two experimental groups of control and diabetic rats. Each group was further subdivided into two subgroups of 5–7 animals based on the acute protocol. These subgroups consisted of rats in which the renal nerves were maintained intact and one in which the kidney had been subjected to acute unilateral renal denervation. A summary of the experimental procedures performed on our study groups is provided in Figure 1.

**Induction of Diabetes Mellitus and Metabolic Studies**

As described previously, the rats were caged individually in custom-built stainless-steel metabolic cages and acclimatized for at least 3 days before the induction of DM by STZ. Baseline physiological data, which comprised body weight, 24-hr water intake, and 24-hr urine output, were recorded on day 1. Subsequently, diabetes was induced using a single i.p. injection of STZ (55 mg/kg) after at least 12 hr of food deprivation. Control cohorts, in contrast, received a single i.p. injection of the vehicle alone. Further physiological data were collected on day 7 before the animals were used in the acute study on day 8. Tail vein blood samples were also collected on days 1 and 7.

The diabetic state in rats was confirmed on day 3 by measuring fasting blood glucose (FBG). Blood was withdrawn from the tail (9:00–9:30 a.m.) and tested for glucose level using a glucometer (ApexBio, Hsinchu, Taiwan). Rats with an FBG level of >250 mg/dl (13.8 mmol/L) were considered diabetic, while STZ-treated rats with a lower glucose level were excluded from the study. Early renal impairment in the diabetic rats was evaluated in terms of FENa, PNa, GFR, and PCr.

**Animal Surgical Preparation for Renal Functional and Hemodynamic Studies**

Rats were food restricted the night before use and given free access to water. Anesthesia was induced by sodium
pentobarbitone (Nembutal®, CEVA Santé Animale, Libourne, France) given at a dose of 60 mg/kg (i.p.). A tracheotomy (PE250, Portex, Kent, UK) was carried out to facilitate spontaneous respiration. A polyethylene catheter (PE50, Portex) was inserted into the right carotid artery for blood sample collection and direct measurement of mean arterial pressure (MAP) by means of a pressure transducer (P23 ID Gould, Statham Instrument, Nottingham, UK) connected to a computerized data acquisition system (PowerLab®, ADInstruments, Sydney, Australia). In order to compensate for fluid losses during the procedure, 0.9% (w/v) isotonic saline was infused at 6 ml/hr (i.v.) throughout the experiment. Supplementary doses of pentobarbitone (10 mg/kg in saline) were given i.v. as required. The left kidney and its renal artery were exposed via a midline abdominal incision. For clearance studies, a cannula (PE10, Portex) was inserted into the left ureter for urine sample collection. Thereafter, a 2-ml bolus of isotonic saline was given i.v. and experiments began 1 hr later.

ARD of the left kidney was then performed by stripping the renal artery and vein out of its adventitia followed by coating the remaining tissue with a solution of 10% phenol in absolute alcohol. In the control and untreated diabetic littermates, the renal sympathetic nerves were left intact and the animals were allowed an equivalent time period (~15 min) before commencing the experimental protocols.

In the renal hemodynamic study, a flowmeter probe (EP 100 series, Carolina Medical Instrument, King, NC) was attached to the dissected left renal artery. The probe was then connected to a Square-Wave Electromagnetic flowmeter (Carolina Medical Instrument) which was linked to a computerized data acquisition system (PowerLab®, ADInstruments).

### Experimental Protocols

#### Protocol I: Renal functional study

Six 20-min urine collections were performed for measurement of fractional sodium excretion (FE\textsubscript{Na}) and glomerular filtration rate (GFR). Blood samples were obtained at the same time intervals for measurement of plasma sodium (P\textsubscript{Na}) and creatinine (P\textsubscript{Cr}), which were then used to calculate FE\textsubscript{Na} and GFR, respectively. Urine collections were not carried out until 5 min after the denervation procedure in order to clear preformed urine from the ureteral cannula.

#### Protocol II: Renal hemodynamic study

The renal hemodynamic study aimed to assess the vasoconstrictor responses associated with the stimulation of the renal nerves in an attempt to characterize the effect of neurally released norepinephrine (NE) on the renovascular responsiveness and renal hemodynamics of SD rats with or without STZ-induced DKD. As we previously described,21–25 MAP was continuously monitored. Baseline RVR and RBF were determined before commencing renal nerve stimulation (RNS) experiment. The renal nerves were stimulated (Grass S 48 Stimulator, Grass Instruments, MA) using a custom-made stainless steel bipolar electrode at frequencies of 1, 2, 4, 6, 8, and 10 Hz (at 0.2 msec duration and 15 V for a period of 15 sec) and then in the reverse order of the same frequencies. Changes in RVR along with the magnitudes of reduction in RBF in response to the nerve stimulation were continuously recorded. Prior to testing at each frequency, RVR and RBF were allowed to return to the baseline prestimulus values. In the denervated animals the stimulation was carried out at the distal cut end of the renal nerve from the left kidney hilus.

### Biological Samples and Biochemical Analyses

Urine samples were collected in microcentrifuge tubes (Eppendorf, Hamburg, Germany) and the volumes obtained were quantified gravimetrically. Blood samples were withdrawn (0.5 ml) from the tail vein during the metabolic studies and the right carotid artery during the acute protocol. Blood was collected in precooled heparinized tubes, centrifuged (3,000 rpm, 1 min) and the clear plasma was separated. During the acute studies, the blood cells were resuspended in saline at an equal volume to the plasma obtained and reinfused into the animal immediately. Plasma and urine samples were stored at −4°C until assayed for sodium using flame emission photometry (Hitachi, Japan) and creatinine by means of a standard colorimetric procedure (Jaff Method, Roche, Switzerland).

### Calculations, Presentation, and Statistical Analyses of Data

The renal functional responses represent the average of six clearances obtained from protocol I. Variables from the metabolic cage experiments and the renal functional study were calculated as follows:

- **Urine flow (UV),** UV = V/T × BW. Here, UV is the urine flow rate, V is the urine volume, T is the time, and BW is the body weight of the rat.
- **Creatinine clearance (Cr),** Cr = U\textsubscript{Cr} × BW/P\textsubscript{Cr}, where U\textsubscript{Cr} is the clearance of creatinine.

Fractional excretion of sodium (FE\textsubscript{Na}) was calculated by C\textsubscript{Na}/GFR, where C\textsubscript{Na} is the clearance of sodium.

The vasoconstrictor responses caused by RNS were taken as the average values caused by each frequency applied across the two ranges (ascending and descending). The data on the drop of RBF were expressed as percentage drop in RBF in relation to the basal RBF values calculated at the beginning of the administration of each stimulation frequency used. The findings on RVR were derived using the equation: 

\[ \text{RVR} = \frac{\text{MAP}}{\text{RBF}} \]

### RESULTS

#### Characteristics of Experimental Animals

Table I presents the physiological parameters form the diabetic and control groups subjected the metabolic studies. After 7 days following onset of DM induced by STZ, diabetic rats manifested a marked loss in body mass, polydipsia (increased daily water intake), and polyuria (increased urine flow rate) (all P < 0.05 vs. baseline measurements prior to STZ administration). As depicted in Table II, renal functional markers measured during the metabolic cage experiments showed a significantly (all P < 0.05) lower FE\textsubscript{Na} but concomi-
diabetic rats however, did not significantly alter $P_{\text{Na}}$ as compared to the innervated control group. In denervated STZ-induced diabetic rats, on the other hand, there was a significantly lower ($P < 0.05$) $P_{\text{Na}}$ relative to the innervated littermates. Conversely, denervated diabetic rats showed a measurably higher ($P < 0.05$) $P_{\text{Na}}$ when compared to the denervated control group.

Effects on GFR (Fig. 2C) A prominent increase ($P < 0.05$) in GFR was observed in diabetic rats with intact renal innervation as compared to the innervated control counterparts. Likewise, there was a significantly higher ($P < 0.05$) GFR in control rats induced with ARD relative to rats with intact renal nerves. Interestingly, compared to innervated diabetic rats and denervated control animals, denervated STZ-induced diabetic animals had significantly lower ($P < 0.05$) GFR measurements.

Effects on $P_{\text{Cr}}$ (Fig. 2D) In the absence of ARD, there was a markedly higher ($P < 0.05$) $P_{\text{Cr}}$ in STZ-induced diabetic rats compared to the respective control. $P_{\text{Cr}}$ measurements were relatively comparable in control rats with or without renal denervation. In denervated diabetic rats, by contrast, ARD resulted in a significantly lower ($P < 0.05$) $P_{\text{Cr}}$ in diabetic rats as compared to the innervated cohorts. Furthermore, the reduction in $P_{\text{Cr}}$ of denervated diabetic rats brought the measurements to comparatively similar values to the ones observed with the denervated control littermates.

Effects of STZ-Induced Diabetes and Renal Denervation on Hemodynamic Responses

Systemic and renal hemodynamic parameters for all experimental groups are shown in Figure 3, while Figure 4 shows the effect of RNS on RVR and the percentage drop in RBF.

Effects on MAP (Fig. 3A) Per se diabetes and/or renal denervation in rats did not significantly alter MAP in all experimental groups.

Effects on baseline RVR (Fig. 3B) In general, baseline RVR measurements were relatively comparable in all study groups. Despite the fact that STZ-induced diabetic rats, irrespective of the innervation status, tended to have lower RVR relative to the control littermates, these levels did not attain statistical significance.

TABLE II. Biochemical and Renal Functional Parameters From the Metabolic Study in Control and Streptozotocin-Induced Diabetic Sprague–Dawley Rats

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Days</th>
<th>$\text{FE}_{\text{Na}}$ (%)</th>
<th>$P_{\text{Na}}$ (mmol/L)</th>
<th>GFR (ml/min/kg)</th>
<th>$P_{\text{Cr}}$ (mg/dl/kg)</th>
<th>FBG (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control rats 1</td>
<td>1</td>
<td>0.6 ± 0.1</td>
<td>144.7 ± 3.7</td>
<td>4.5 ± 1.0</td>
<td>6.7 ± 0.1</td>
<td>930 ± 4.5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Not done</td>
<td>Not done</td>
<td>Not done</td>
<td>Not done</td>
<td>870 ± 4.0</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.7 ± 0.1</td>
<td>148.3 ± 4.4</td>
<td>4.1 ± 0.9</td>
<td>7.0 ± 0.1</td>
<td>Not done</td>
</tr>
<tr>
<td>Diabetic rats 1</td>
<td>1</td>
<td>1.0 ± 0.2</td>
<td>140.8 ± 6.6</td>
<td>5.0 ± 1.1</td>
<td>5.9 ± 0.1</td>
<td>925 ± 5.8</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Not done</td>
<td>Not done</td>
<td>Not done</td>
<td>Not done</td>
<td>401.6 ± 30.3*</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.3 ± 0.1*</td>
<td>162.0 ± 3.3*</td>
<td>11.4 ± 1.4*</td>
<td>7.7 ± 0.1*</td>
<td>Not done</td>
</tr>
</tbody>
</table>

$\text{FE}_{\text{Na}}$, fractional excretion of sodium; $P_{\text{Na}}$, plasma sodium; GFR, glomerular filtration rate; $P_{\text{Cr}}$, plasma creatinine; FBG, fasting blood glucose.

Data are presented as mean ± SEM (all $n = 5–7$). All data were analyzed by one-way ANOVA followed by Bonferroni–Dunn (all mean) post hoc test. All data in Table II were previously described by Salman et al.20 except for the baseline measurements of FBG in the diabetic group.

*P < 0.05 versus baseline value on day 1 in the same experimental group prior to the induction of diabetes in the diabetic group or the administration of the vehicle in the control group.

Neurourology and Urodynamics DOI 10.1002/nau
Effects on baseline RBF (Fig. 3C) Likewise, baseline RBF measurements did not vary significantly in all study groups. However, in contrast to the findings on RVR, a tendency towards higher RBF was observed in diabetic rats compared to other experimental groups. Nonetheless, the effects did not reach the level of statistical significance.

Effects of RNS on RVR (Fig. 4A) The application of a direct electrical stimulus to the renal sympathetic nerves resulted in frequency-dependent increases in RVR in all experimental groups. However, the overall increase in RVR was significantly \( P < 0.05 \) lower in diabetic rats with intact renal innervation compared to the respective control group, especially at lower frequencies. A markedly lower \( P < 0.05 \) renal vasoconstrictor response to RNS, as reflected by the reduction in RVR measurements, was observed in both control and diabetic rats following the induction of ARD. Interestingly, denervated STZ-induced diabetic rats exhibited significantly higher \( P < 0.05 \) RVR responses against RNS as compared to the denervated control group.

The overall changes in RVR and RBF in response to direct electrical stimulation of renal sympathetic nerves are summarized in Table III.

DISCUSSION

The role of renal sympathetic nervous system in the pathogenesis of STZ-induced DKD has been largely unexplored. Therefore, the present study aimed to address this issue in an attempt to further clarify the functional contribution of renal sympathetic nervous system in the deterioration of renal function during early course of STZ-induced DKD. Data reported herein provided evidence that renal nerves are functionally involved in the intrarenal hemodynamic and functional abnormalities observed in the early stages of experimental DM. Furthermore, results from the present work suggested that several manifestations of renal function and hemodynamics can be substantially alleviated when renal denervation is performed under this pathophysiological condition.

During the metabolic cage experiments in diabetic rats, there was a significant increase in FBG levels which was accompanied by classical polyuria, polydipsia, and massive weight reduction. These findings are in agreement with several earlier reports on the characteristic signs and reduction in RBF, in denervated control and diabetic rats relative to the innervated cohorts. Surprisingly, ARD in STZ-induced diabetic rats contributed to a significantly higher \( P < 0.05 \) percentage drop in RBF in denervated diabetic rats as compared to the denervated control group.
symptoms of DM. The early (7 days following the induction of STZ-induced diabetes) significant reduction in sodium excretion (FENa < 1%) and the considerable increases in PCr and GFR (glomerular hyperfiltration) were perhaps indicative of prerenal acute renal dysfunctions as described by previous investigators and a recent study from our laboratory.

Data derived from the acute protocol interestingly showed that ARD possessed a considerable ameliorative efficacy against the compromised renal function accompanying recent onset DM. In general, there was a substantial effect for renal denervation on GFR. The other parameters were statistically significant, but numerically not marked. Nonetheless, the numerically smaller effects observed in our experiments were of apparent physiological significance when correlated with the normal ranges of the renal functional markers studied.

In the setting of diabetic rats with intact renal nerves, we have observed markedly low FENa which came in tandem with a significantly high PNa. Together, these observations would confirm an apparent renal impairment of sodium handling.

Previous studies have shown that the development of DKD is related to an enhancement in the proximal Na\(^+\)–K\(^+\)/ATPase activity and/or Na\(^+\)–glucose co-transport, an effect contributes to increased proximal tubular sodium reabsorption. It has further been reported that stimulation of postsynaptic \(\alpha_1\)-adrenergic receptors increases tubular Na\(^+\)–K\(^+\)/ATPase activity leading to an apparent reduction of sodium. Our results clearly suggest a role for renal sympathetic nerves in the mediation of impaired renal handling of sodium since the removal of renal sympathetic tone significantly improved sodium responses in diabetic rats.

A possible explanation for the mechanism of this effect is the reduction in the sympathetically mediated activation of tubular Na\(^+\)/K\(^+\)/ATPase in response to the denervation-induced cessation of presynaptic noradrenergic neurotransmission. However, this issue remains to be investigated in further experiments.

Further evidence in support of the stated view that renal adrenergic neurons are primarily involved in the renal functional disturbances accompanying DKD came from the observation that the induction of renal denervation in this pathological state markedly improved glomerular hyperfiltration and PCr. However, the possible mechanisms involved in these effects remain to be determined. Despite the fact that innervated diabetic animals showed a tendency towards increased RBF and reduced RVR as compared to the non-diabetic control counterparts, a characteristic of glomerular hyperfiltration, the basal renal hemodynamic parameters were statically comparable with or without renal denervation. Therefore, one possibility behind the observed changes in GFR is that the TGF system senses changes in the concentration of Na\(^+\) at the luminal macula densa and induces reciprocal changes in GFR. TGF stabilizes electrolyte delivery to the distal tubule which in these nephron segments allows fine adjustment of reabsorption and excretion according to bodily needs. A primary increase in proximal reabsorption reduces the concentration of salt at the macula densa. This elicits a TGF-dependent increase in GFR, which partially compensates for the impact of the original disturbance on macula densa delivery. Therefore, attenuation of the hyperactive renal sympathetic nerve functions in diabetic rats using ARD could have increased distal tubular Na\(^+\) delivery to the macula densa resulting in TGF-dependent reduction in GFR.

It is widely acknowledged that under normal physiological condition, basal renal nerve activity, and mechanico-chemical renal denervation do not influence renal hemodynamics. Results of the present study are in agreement with these reports showing that MAP, basal RVR, and basal RBF were similar while comparing control rats prior to and after renal denervation. Furthermore, our study extended these findings to diabetic rats.
findings to show comparable hemodynamic measures in both innervated and denervated rats with recent onset STZ-induced diabetes. Thus, it is important to emphasize that the differences in the renal functional parameters or the dynamic autoregulation of RBF and RVR in response to RNS were unlikely to be due to major differences in systemic MAP as it was not significantly affected by the disease state, renal denervation or saline maintenance infusion in any of the groups. It may be argued that diabetic rats could be viewed as under-hydrated since the fluid input rate was similar for both control and diabetic animals (6 ml/hr). Considering our objectives, which collectively endeavored to assess the pathophysiological impact of recent onset DM on the renal sympathetic nerve function, it would not have been appropriate if we had totally corrected the dehydration state in STZ-induced diabetic rats. Otherwise, the establishment of a complete euhydration state in diabetic rats could have masked the pathological effects of diabetes, its impact on the renal sympathetic nerve function and the overall assessment of the renal functional and hemodynamic parameters.

In the present study, evident attenuation in the renal vasoconstrictor responses to direct electrical stimulation of renal efferent nerve, as reflected by the changes in RVR and percentage of reduction in RBF measured 7 days after the induction of diabetes in rats, was observed. These findings suggested an early renal sympatho-excitation and the possible existence of augmented presynaptic NE release from adrenergic nerve terminals and/or reduced neuronal uptake. It has been reported that augmented sympathetic nervous system activity and prolonged exposure to catecholamines can contribute to reduced adrenoceptors density and/or dampening of receptor responsiveness to endogenous and exogenous adrenergic vasoconstrictors, a phenomenon known as desensitization. Desensitization is a complex process mediated through a number of inter-related yet distinct mechanisms occurring at the receptor level to turn off the receptor-mediated signal transduction pathway. While the G-protein coupled 2-adrenoceptors are predominantly responsible for the regulation of renal vascular tone, it can be suggested that the observed reduction in renal vasoconstrictor responses to RNS could have been a direct consequence of desensitization of this receptor subtype by the hyperactive renal sympathetic tone present in this pathophysiological condition. Despite the fact that basal RVR and RBF were statistically comparable to those of the control, the presence of impaired renal vasoconstrictor responses to RNS was very much indicative that enhanced noradrenergic neurotransmission and possible action on postsynaptic 2-adrenoceptors could have, at least partly, mediated the early deterioration of renal hemodynamic and excretory functions in diabetic rats.

Neurourology and Urodynamics DOI 10.1002/nau

<table>
<thead>
<tr>
<th>Table III. Overall Mean Change in the Hemodynamic Responses to Renal Nerve Stimulation During the Acute Renal Hemodynamic Study in Control and Streptozotocin-Induced Diabetic Sprague–Dawley Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall changes in the hemodynamic responses to RNS</td>
</tr>
<tr>
<td>Experimental group</td>
</tr>
<tr>
<td>INN-C</td>
</tr>
<tr>
<td>DNX-C</td>
</tr>
<tr>
<td>INN-DM</td>
</tr>
<tr>
<td>DNX-DM</td>
</tr>
</tbody>
</table>

| Experimental group                  | RVR (mmHg/ml/min) | | in RBF (%) |
| INN-C                        | 24.7 ± 0.6*       | 54.1 ± 3.3 |
| DNX-C                        | 17.7 ± 0.4*       | 17.6 ± 3.4 |
| INN-DM                      | 21.7 ± 0.6*       | 44.3 ± 3.1* |
| DNX-DM                      | 19.3 ± 0.4*       | 23.4 ± 1.5* |

| Experimental group                  | RVR (mmHg/ml/min) | | in RBF (%) |
| INN-C                        | 24.7 ± 0.6*       | 54.1 ± 3.3 |
| DNX-C                        | 17.7 ± 0.4*       | 17.6 ± 3.4 |
| INN-DM                      | 21.7 ± 0.6*       | 44.3 ± 3.1* |
| DNX-DM                      | 19.3 ± 0.4*       | 23.4 ± 1.5* |

*P < 0.05: significant difference between DNX-C and INN-C.
**P < 0.05: significant difference between INN-DM and INN-C.
***P < 0.05: significant difference between DNX-DM and INN-C.
****P < 0.05: significant difference between DNX-DM and DNX-C.

Fig. 4. Renal nerve stimulation (RNS) responses in innervated control (INN-C), denervated control (DNX-C), innervated diabetic (INN-DM), and denervated diabetic (DNX-DM) Sprague–Dawley rats: (A) effect on renal vascular resistance (RVR) and (B) effect on percentage reduction in renal blood flow (RBF). Data are presented as mean ± SEM (n = 5–7). *P < 0.05: significant difference between INN-DM and INN-C. †P < 0.05: significant difference between DNX-DM and INN-DM. \( \frac{5}{4} P < 0.05: \) significant difference between DNX-DM and DNX-C. Data are presented as mean ± SEM (n = 5–7). Data were analyzed by two-way ANOVA followed by Bonferroni–Dunn (all means) post hoc test.20–23,36 Receptor desensitization may take place within seconds to minutes of agonist stimulation, and is commonly considered to result from receptor uncoupling from downstream effectors due to receptor phosphorylation.21,22,36 While the G-protein coupled 2-adrenoceptors are predominantly responsible for the regulation of renal vascular tone, it can be suggested that the observed reduction in renal vasoconstrictor responses to RNS could have been a direct consequence of desensitization of this receptor subtype by the hyperactive renal sympathetic tone present in this pathophysiological condition. Despite the fact that basal RVR and RBF were statistically comparable to those of the control, the presence of impaired renal vasoconstrictor responses to RNS was very much indicative that enhanced noradrenergic neurotransmission and possible action on postsynaptic 2-adrenoceptors could have, at least partly, mediated the early deterioration of renal hemodynamic and excretory functions in diabetic rats.

Neurourology and Urodynamics DOI 10.1002/nau
On the other hand, the ameliorative effects of renal denervation on renal function and hemodynamics were unlikely to be due to an effect other than that of renal denervation-induced reduction in renal sympathetic outflow since a marked drop in the renal vasoconstrictor response to RNS was observed when the stimulation was performed in denervated animals. The latter observation indicated the efficiency of this procedure in establishing a sufficient level of peripheral sympato-inhibition in the diseased animals.

With respect to the effect of ARD on the renal vasculature, it is noteworthy to highlight the issue of α1-adrenoceptors hyperresponsiveness to catecholamines. Studies comparing the effect of catecholamines on renal hemodynamics in innervated and denervated kidneys propose that chronically (7–10 days) denervated kidneys exhibit supranormal sensitivity to NE. By contrast, Ramchandra et al. (2000) suggested that the denervated kidney does not become supersensitive to physiological levels of circulating NE. In the light of the present results, enhanced adrenoceptors responsiveness to catecholamines in the renal vasculature could have occurred. To our knowledge, this observation in acutely denervated kidney of diabetic rats has not been reported by other investigators before. To this end, the application of a direct electrical stimulus in the denervated diabetic rats at a site distal to the position of dissection but close to the left kidney interestingly showed significant increases in RVR and the percentage drop in RBF compared with their denervated control counterparts. While the renal denervation procedure used in our experiments does not permit a specific targeting of the intrarenal branches of the efferent renal sympathetic nerve, we mainly attribute these observations to enhanced adrenoceptors sensitivity to remnants of intrarenal NE triggered by the electrical stimulus. This proposition holds true when the denervation procedure was performed on acute basis before an ongoing depletion of NE pool in the presynaptic nerve terminals was observed in response to the denervation-induced abrogation of the thoracolumbar sympathetic outflow supplying the kidney. The application of the electrical stimulus could have compensated for the denervation-induced loss in the repetitive action potential firing from the renal sympathetic nerve supply from the spinal cord. In other words, the electrical stimulus could have triggered the influx of calcium ions inside the intrarenal presynaptic nerve endings causing the fusion of the NE-laden synaptic vesicles with the presynaptic membrane and, thereafter, an enhancement in NE release. Several lines of evidence have suggested a role for both pre- and postsynaptic mechanisms in the development of hypersensitivity. The presynaptic mechanism comprised the loss of NE uptake into the renal sympathetic nerve terminals. The up-regulation of the postsynaptic adrenoceptors due to reduced local NE release has been proposed as a postsynaptic mechanism. However, these mechanisms warrant further investigation.

CONCLUSIONS

The present data convincingly show that renal sympathetic nerves are functionally involved in the pathogenesis of DKD. An enhanced impact of the renal sympathetic nerves on excretory function is an early consequence of this pathophysiological condition. ARD considerably improves the pattern of renal dysfunctions in rats with recent onset DKD. The data further suggest an early enhancement in renal sensitivity to intrarenal NE upon the removal of renal sympathetic tone in STZ-induced diabetic rats.

Role of Renal Nerves in Diabetic Kidney Disease

To date, there is no effective treatment for DKD and diabetic nephropathy. Our data demonstrate the presence of sympatho-hyperreactivity as a critical rate limiter of disease progression, thus highlighting the complexity of DKD and the need to develop multi faceted pharmacological interventions. Therefore, future therapeutics should aim to correct the state of renal sympatho-excitato in diabetic patients and to apply these measures as soon as the disease is diagnosed.

ACKNOWLEDGMENTS

Ibrahim M. Salman is a beneficiary of Universiti Sains Malaysia fellowship award.

REFERENCES


