Renal functional & haemodynamic changes following acute unilateral renal denervation in Sprague Dawley rats


Background & objectives: Regulation of renal function and haemodynamics are under a direct control from the renal sympathetic nerves and renal denervation produces overt diuresis and natriuresis in several mammalian species. However, the inter-related series of changes in renal function and haemodynamics following acute renal denervation (ARD) is not fully understood. Thus, we aimed to investigate and relate the changes in renal function and haemodynamics following acute unilateral renal denervation in anaesthetized Sprague Dawley (SD) rats.

Methods: Male SD rats were fasted overnight, anaesthetized with sodium pentobarbitone (60 mg/kg ip), denervated by application of phenol to the left renal artery and maintained on an intravenous (iv) infusion of isotonic saline for 2 h. Throughout this period, six urine and plasma samples were taken at 20-min intervals to study kidney function parameters. In a different set of experiments, renal nerve stimulation (RNS) was carried out to characterize the changes in renal vasoconstrictor responses following ARD.

Results: Denervated animals showed significantly (P<0.05 vs. control innervated rats) higher urine flow rate (UFR), absolute sodium excretion (\(U_{Na}\)), fractional sodium excretion (\(FE_{Na}\)) and glomerular filtration rate (GFR). The renal vasoconstrictor responses to RNS were significantly (P<0.05) lower in denervated rats as compared to the innervated counterparts. However, no appreciable differences were seen in the mean arterial pressure (MAP), plasma sodium (\(P_{Na}\)), basal renal blood flow (RBF) and basal renal vascular resistance (RVR) in both innervated and denervated SD rats.

Interpretation & conclusions: Natriuresis, diuresis, enhanced GFR and impaired vasoconstriction in response to RNS are typical and instant responses to ARD in SD rats. Renal sympathetic nerves serve more important role in salt and water conservation than in dynamic autoregulation of RBF under normal sympathetic tone; yet, their effects on renal haemodynamics become more evident in the presence of augmented renal sympathetic nerve activity (RSNA).

Key words Acute renal denervation - diuresis - natriuresis - renal artery - renal nerve - renal blood flow - Sprague Dawley rats
Research in the area of the neural control of kidney functions was not vigorous during the first half of the 20th century because only a few functional consequences were attributed to changes in renal nerve function based on experiments involving denervation in anaesthetized and surgically operated animals. Smith disapproved this approach, as he believed that the starting level of renal sympathetic nerve activity (RSNA) would be quite elevated due to the associated stress. Further, there was a lack of convincing evidence that the renal innervation extended beyond the renal vasculature to either the tubules or the juxtaglomerular apparatus.

Renal sympathetic outflow to the kidneys plays a pivotal role in the regulation of fluid and electrolytes in the body and, therefore, mean arterial pressure (MAP) homeostasis. The renal sympathetic nerves enter the hilum of the kidney with the renal artery and the renal vein and thereafter are distributed along the renal arterial segments in the renal cortex and outer medulla. Fluctuations in the degree of RSNA modulate renin secretion from juxtaglomerular cells and sodium reabsorption from renal tubular cells. There is evidence indicating the involvement of renal adrenergic nerves in the regulation of renal vascular resistance (RVR) and, ultimately, renal haemodynamics.

A popular way for discerning the role of the renal sympathetic nerves in the regulation of renal function and haemodynamics is to denervate one kidney. Renal denervation is well reported to produce an increase in sodium and water excretion in several mammalian species. Also, denervation in normal rats does not influence renal haemodynamics. However, a considerable controversy still exists on some aspect of the instant changes in renal haemodynamic and excretory functions following acute renal denervation (ARD).

The present study was undertaken to further investigate the changes in renal function and haemodynamics in rats subjected to acute unilateral renal denervation in an attempt to functionally characterize the sympathetic nerve control of the kidney. To study the association between the changes in renal haemodynamics and excretory function, several biochemical parameters were measured and the changes in basal renal haemodynamic parameters and renal vasoconstrictor responses to renal nerve stimulation (RNS) were recorded.

**Material & Methods**

*Animals:* Adult male SD rats weighing 250-300 g were obtained from the Animal Care Facility, Universiti Sains Malaysia (USM), Penang, Malaysia. The animals were housed in standard environmental conditions (25°C, 60-70% humidity) under natural lighting and fed with normal commercial rat chow and water ad libitum. They were also allowed to acclimatize in the animal transit room for a minimum period of one week before being used for any experiment. Animals handling and all procedures on animals were carried out in accordance with the guidelines of the Animal Ethics Committee, USM, Penang, Malaysia. After acclimatization of a week, animals were randomly divided into 2 groups. Group 1 was used to carry out the renal functional study while group 2 was employed to perform the renal haemodynamic study. Each group was further subdivided into denervated and control innervated subgroups (n = 6 in each group).

*Surgical preparation of animal:* The rats were fasted overnight with access to drinking water ad libitum. Anaesthesia was induced by sodium pentobarbitone (Nembutal®, CA VE, France) at a dose of 60 mg/kg (ip). After a tracheotomy (PE250, Portex, UK), the left jugular vein cannulation was performed to enable the administration of an intravenous (iv) maintenance infusion of saline (0.9% NaCl solution) at an infusion rate of 6 ml/h and to allow an intermittent administration of supplementary bolus injections of anaesthetic (10 mg/kg in isotonic saline). The right carotid artery was catheterized (PE50, Portex, UK) for blood sample collection and/or direct measurement of 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). A midline abdominal incision was carried out to expose the left kidney. Upon completion of the animal surgical preparation, 2 ml of saline (iv) were given to the animal, and then left for a period of 1 h to stabilize.

*Acute renal denervation (ARD):* The isolated left renal artery was subjected to ARD. The procedure was performed by stripping off the renal artery and vein out of its adventitia. All observable renal nerves passing from the celiac and aortico-renal ganglia to the kidney were carefully isolated, dissected and cut. This was followed by coating of the remaining tissue with a solution of 10 per cent phenol in absolute alcohol. In the control animals, the renal nerve was left intact and the animal was allowed to stand for an equivalent time period before commencing the experimental protocols.

The loss of the functions of the renal nerves was tested by stimulating them (Grass S48 stimulator,
Grass instrument, MA, USA) at 15 V, 0.2 msec, 1-4Hz for 15-30 sec. Blanching of the kidney in response to electrical stimulation, which is usually observed in the intact renal nerves, was completely lost after renal denervation.

Experimental protocols: Once renal denervation was established, a period of 30-45 min was allowed and the animals were subsequently subjected to one of the following protocols:

Protocol I (n=12): The left ureter was cannulated (PE10, Portex, UK). Six urine collections were performed at 20-min intervals for 2 h to measure urine volume and subsequently calculate the urine flow rate (UFR), absolute sodium excretion (U_{Na}V) and fractional sodium excretion (FE_{Na}) and glomerular filtration rate (GFR). Blood samples were obtained at the same time intervals for measurement of plasma sodium (P_{Na}) and creatinine (P_{Cr}) and then calculating FE_{Na} and GFR, respectively.

Protocol II (n=12): Basal RBF and RVR values were initially determined. Subsequently, the renal nerves were stimulated (Grass S 48 Stimulator, Grass Instruments, MA, USA) at increasing frequencies of 1, 2, 4, 6, 8, and 10 Hz, at 0.2 msec duration and 15 V for a period of 15 sec in ascending and then descending order. Changes in RBF and RVR in response to electrical stimulation of the renal nerve were measured by placing a flowmeter probe (EP 100 series, Carolina Medical Instrument, King, North Carolina, USA) on the isolated renal artery. The probe was connected to a Square-Wave electromagnetic flowmeter (Carolina Medical Instrument, King, North Carolina, USA) which was linked to a computerized data acquisition system (PowerLab®, ADInstruments, Australia).

At the end of the experiment, the animals were killed by an overdose of anaesthesia and disposed of in accordance with the guidelines of the Animal Ethics Committee of USM, Penang, Malaysia.

Analytical procedures: Urine samples were collected in microcentrifuge tubes (Eppendorf, Hamburg, Germany) and the volumes obtained were gravimetrically quantified. Blood samples were collected (0.5 ml) from the right carotid artery into a pre-cooled heparinized syringe, centrifuged and the clear plasma was separated. 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 photometry (Hitachi, Japan) and creatinine by spectrophotometry (Hitachi, Japan).

Calculations: Urine flow rate (UFR) was calculated by the following formula: UFR = UV / T x BW. Here, UV is the urine volume, T is the time and BW is the body weight of the rat.

Absolute excretion of sodium (U_{Na}V) was calculated using the equation: U_{Na}V = U_{Na} x UFR. Here, U_{Na} is the urine concentration of sodium.

Clearances were calculated using the usual formula: C_{x} = U_{x} x UFR / P_{x}, where, C_{x} is the clearance of substance x and P_{x} is the plasma concentration of x. Glomerular filtration rate (GFR) was taken to be the clearance of creatinine. Fractional excretion of sodium (FE_{Na}) was calculated by C_{Na}/GFR, where C_{Na} is the clearance of sodium.

Renal vascular resistance (RVR) was calculated by the equation: RVR = MAP/RBF, where MAP is the mean arterial pressure and RBF is the renal blood flow.

Statistical analysis: All data were expressed in terms of mean ± SEM. The statistical analysis of the data was done using one- and two-way ANOVA followed by Bonferroni/Dunnett (all mean) post hoc test using Superanova statistical package (Abacus Inc., Barkley, CA, USA). One-way ANOVA was used to compare rats’ body weight and the basal values of RBF and RVR. Alternatively, changes in the overall mean of the functional parameters and haemodynamic responses to RNS were compared by two-way ANOVA. The differences between the means were considered significant at 5 per cent level.

Results

Body weights of the rats were similar in the two groups (control innervated group: 288.0 ± 4.5 and denervated group: 280.4 ± 5.7 g).

Effects of acute renal denervation (ARD) on renal function: In the present study, ARD produced a significantly (P<0.05) higher UFR measured at 20-min intervals for 2 h as compared with that measured at the same time points in rats with intact renal innervation (Fig. 1A). Like UFR, it was further observed that the U_{Na}V and FE_{Na}, which were measured using the same renal clearance protocol, were significantly (all P<0.05) higher in the denervated animals as compared to those measured at the same time intervals in innervated counterparts (Figs. 1B & 1C). Conversely,
remained constant in both experimental groups (Fig. 1D). In denervated rats, however, a significantly ($P<0.05$) higher GFR was observed as compared to the control (Fig. 1E).

**Effects of acute renal denervation (ARD) on haemodynamic responses:** There was no significant difference in MAP of denervated and innervated animals (Fig. 2A). In denervated rats, there were no significant changes in the basal values of RBF (denervated group: 18.0 ± 1.1 ml/min/kg vs. control innervated group: 17.6 ± 1.2 ml/min/kg) and RVR (denervated group: 21.9 ± 1.4 mmHg/min/kg vs. control innervated group: 23.0 ± 2.6 mmHg/min/kg) compared to rats with intact renal nerves. However, there was a frequency-dependent reduction in RBF and concomitant frequency-related increase of RVR in all experimental groups. It was observed that the overall mean values of percentage reduction in RBF and RVR were significantly ($P<0.05$) lower in the denervated rats as compared to innervated control counterparts (Figs 2B & 2C).

**Discussion**

The major finding of the present study is the demonstration that ARD-induced diuresis and natriuresis can possibly be accompanied by a prominent enhancement in whole kidney GFR even when basal renal haemodynamic responses remain unaltered. Further, the effect of norepinephrine (NE) spillover from renal adrenergic neurons on salt and water conservation outweighs its effect on renal haemodynamics under normal sympathetic tone. Yet, the latter effect becomes more pronounced under hyperactive renal sympathetic nervous system.

In our study, instant significant changes in salt and water excretion were seen directly after renal denervation. Approximately three-fold higher UFR, $U_{Na}V$ and $FE_{Na}$ were observed in the denervated animals compared to rats with intact renal nerves. The observed diuresis and natriuresis following removal of renal sympathetic tone have been reported in studies from our research group and several other studies on acute and chronic renal denervation$^{17,18,24,25}$. Together, these results suggested and further confirmed that renal sympathetic activity is involved in sodium and water regulation. It is noteworthy to mention that it is unlikely that the observed diuresis and natriuresis were due to changes other than renal denervation. One might question whether the phenol itself could have directly been responsible for these effects. Such proposition is
systemic effect related to the duration of the experiment, such as extracellular fluid expansion leading to enhanced salt and water excretion, can be excluded since iv fluid input was carefully adjusted to 6 ml/h and there were no alterations in salt and water excretion by the innervated kidney of the control SD rats. SD rats also showed that the enhanced diuretic and natriuretic responses were accompanied by an eminently higher GFR compared to earlier reports of no change in GFR. Therefore, it can be suggested that renal adrenergic neurons play a considerable role in the control of GFR under basal sympathetic tone. While the findings collectively showed no significant effect for renal denervation on basal RBF and RVR, it was unlikely that denervation-induced loss in renal neural input could have caused an enhancement in GFR through altered RBF. One possible explanation for the observed elevation in GFR is that the loss of sympathetic outflow to the kidney might have caused relaxation of the mesangial cells that surround the glomerular capillaries and therefore contributed to an increase in the surface area of the capillaries at any given net filtration pressure. However, further studies need to be done.

The concept that renal nerves are important in setting the basal level of arterial pressure in normal animals has not been supported by the literature. In fact, the results of previous studies, in which basal levels of arterial pressure were measured in intact and denervated animals, are conflicting. This may be due, in part, to the methods used to measure arterial pressure and how these measurements influence basal sympathetic activity. Nevertheless, there is abundant experimental evidence to support the view that ARD in normotensive rats does not affect MAP. These findings are in accordance with our results showing that MAP was almost the same in both denervated and innervated SD rats and was moreover associated with unchanged plasma sodium levels in both experimental groups.

The effect of renal denervation on renal haemodynamic responses is a subject of debate. However, a large body of experimental evidence has indicated that basal renal nerve activity does not influence renal haemodynamics. For instance, in conscious dogs and humans, surgical or pharmacological renal denervation did not affect RBF or RVR as described by previous reports. Our results were very much in line with the views stated in those reports demonstrating the absence of any significant changes in basal RBF and

considered implausible since phenol is known to be a toxic substance leading to the coagulation and necrosis of tissue protein and its injection, even in minimal amounts, into the renal artery results in the immediate collapse of the surface tubules. On the other hand, any

![Fig. 2. Haemodynamic responses in innervated (□) and denervated (▲) Sprague Dawley rats. (A) Mean arterial pressure (MAP), (B) percentage reduction in renal blood flow (RBF) in response to renal nerve stimulation (RNS) and (C) renal vascular resistance (RVR) responses to renal nerve stimulation (RNS). Data presented as mean ± SEM (all n=6). *P<0.05 compared to control. Data were analyzed by two-way ANOVA followed by Bonferroni/Dunnett post-hoc test.]
RVR following the induction of ARD. These findings suggested that renal nerve tonic influence on renal vasculature was very minimal under normal sympathetic nerve activity. That is to say, lack of renal sympathetic tone by denervation did not produce a remarkable renal arteriolar vasodilatation. Together, our observations signified that under normal sympathetic tone the renal sympathetic nerve fibers play a more important role in salt and water conservation than dynamic autoregulation of renal vascular tone and hence RBF.

The present study has further addressed the effect of renal nerves on renal vasculature under conditions of augmented nerve action by electrical stimulation. Stimulation (1-10 Hz) of intact renal nerves produced a pronounced frequency-dependent increase in RVR and a consequent frequency-related reduction in RBF. In contrast, stimulation of the renal nerves following denervation at a position distal to the site of dissection showed a significant reduction in RVR and the overall percentage drop in RBF when compared to the stimulation of intact renal nerves. These findings demonstrated the effectiveness of the denervation method used and further indicated that renal sympathetic nerves play a very crucial role in the regulation of RVR while facing an exaggerated sympathetic outflow and enhanced NE release from nerve terminals.

In summary, the present results validate the importance of renal nerves in the regulation of renal function and haemodynamics. Diuresis and natriuresis following ARD are well described and reproducible phenomena. These effects are of relatively rapid onset and dependent on the exclusion of the neural stimulatory influences. Renal sympathetic nerves appear to serve a more imperative role in salt and water conservation than in dynamic autoregulation of RVR and RBF under normal sympathetic tone. Enhanced GFR responses are possible consequences of renal denervation even when the effects of renal denervation on basal renal haemodynamics are lacking. Finally, renal nerves play a pivotal role in the regulation of RVR and RBF when NE spillover from the nerve terminals is augmented by enhanced RSNA.

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