Quantification of Acute Renal Denervation Diuresis and Natriuresis in Sprague Dawley and Spontaneously Hypertensive Rats

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Abstract: The present study was undertaken to quantify the renal salt and water excretory functions in response to acute unilateral renal denervation in Sprague Dawley (SD) and spontaneously hypertensive (SHR) rats in an attempt to characterize the relative contribution of renal sympathetic nerve activity (RSNA) to renal functional excretory responses in normotensive and hypertensive conditions. Adult male SD and SHR rats were fasted overnight, anesthetized with pentobarbital sodium (60 mg kg⁻¹ i.p.), denervated by application of phenol to the left renal artery and maintained on an intravenous (i.v.) infusion of normal saline for 2 h. Throughout this period, six urine and plasma samples were collected at 20 min intervals to study kidney function parameters. The data showed that there was a significantly higher (p<0.05) amount of sodium and water excretions in the urine of denervated SD and SHR rats as compared to their innervated counterparts. No significant difference in the renal salt and water excretions was seen between innervated SD and SHR rats; however, the difference was significant (p<0.05) following removal of renal sympathetic input. No appreciable changes in the mean arterial blood pressure (MAP) and plasma sodium (P₅₈) were observed in denervated SD and SHR rats as compared to the innervated ones, yet, MAP values were significantly higher (p<0.05) in denervated and innervated SHR rats in comparison to the denervated and innervated SD rats. Moreover, P₅₈ in denervated SHR rats, which was significantly higher (p<0.05) in SHR rats as compared to SD rats prior to renal denervation, tended to approximate the one in denervated SD rats. In conclusion, this study confirmed the significant role played by the renal nerves in the control of renal functions. Diuresis and natriuresis are typical responses to acute renal denervation (ARD) in SD and SHR rats. Enhanced salt and water excretion following ARD in SHR rats suggests high renal sympathetic nerve discharge in these animals and highlights the significant contribution of renal nerves to the genetic model of essential hypertension.

Key words: Diuresis, natriuresis, acute renal denervation, renal sympathetic nerve activity, renal functions

INTRODUCTION

Fluid and electrolyte regulation in the body along with homeostatic control of mean arterial blood pressure (MAP) are major functions of the kidneys. The kidneys are abundantly innervated internal organs and one mechanism by which they are believed to control this homeostatic process is through the action of the renal sympathetic nerves (DiBona, 1982; Moss, 1982; DiBona and Kopp, 1997; Salomonsson et al., 2000) In rats, adrenergic neurons are localized on the cells of afferent and efferent arterioles and renal tubules. Variations in the extent of renal sympathetic nerve activity (RSNA) can subsequently modulate hormone secretions including renin from juxtaglomerular cells, sodium reabsorption from renal tubular cells and eventually renal hemodynamics (DiBona, 1982; Elzbieta et al., 2001).

Renal denervation has been shown to produce overt diuresis and natriuresis in several mammalian species (Elzbieta et al., 2001; Salman et al., 2008), effects which are mainly attributed to a strong reduction in salt and water reabsorption by the proximal convoluted tubule (PCT) of the kidney following denervation, with partial compensation by an elevation in the absolute rate of reabsorption in the loop of Henle, distal convoluted tubule (DCT) and collecting duct (Ichihara et al., 1997).

Many lines of evidence have implicated sympathetically mediated mechanisms in the development
of hypertension in several rat models (Katholi et al., 1980; Sripairojthikoon et al., 1989). In SHR, high sympathetic nerve action has been verified by direct measurement of RSNA (Jacob et al., 2003). The latter fact suggests a role of afferent and efferent renal nerves in the pathogenic process of hypertension (Bello-Reuss et al., 1975).

Since the augmentation in renal excretory function appears to play a vital role in promoting sustained reductions in MAP and are mediated, at least in part, by suppression of RSNA, the renal nerves offer a rational link between alterations in central sympathetic output and renal function that lead to a lowering of blood pressure (Lohmeier et al., 2005). Therefore, in the present study we attempted to quantify the changes in urinary salt and water excretions following acute unilateral renal denervation in Sprague Dawley (SD) and spontaneously hypertensive (SHR) rats to establish a possible correlation between renal functional excretory responses and the differences in the basal RSNA in normotensive and hypertensive conditions.

**MATERIALS AND METHODS**

**Animals:** Adult male SHR and SD rats (250-300 g) were obtained from the Animal Care Facility, Universiti Sains Malaysia (USM), Penang, Malaysia. The animals were housed in a light-controlled room with a 12 h light/dark cycle and were 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 experiments. Animal handling and all experimental protocols on animals were carried out in accordance with the guidelines of the Animal Ethics Committee, USM, Penang, Malaysia and had their approval. After acclimatization of a week, animals were randomly divided into 4 different groups and each group consisted of 6 animals. Group 1 was control SD rats, group 2 was denervated SD rats, group 3 was control SHR rats, whereas group 4 was denervated SHR rats.

**Surgical preparation of animal:** Overnight-fasted rats were anaesthetized with sodium pentobarbitone (Nembutal®, CAVE, France) at a dose of 60 mg kg⁻¹ (i.p.). After a tracheotomy (PE250, Portex, UK), the left jugular vein cannulation was carried out to enable the administration of an intravenous (i.v.) maintenance infusion of isotonic normal saline at an infusion rate of 6 mL h⁻¹ and to allow the intermittent administration of supplementary anesthetic bolus injections (10 mg kg⁻¹ in normal saline). The right carotid artery was similarly catheterized (PE50, Portex, UK) for blood-sample collection and direct measurement of MAP using a pressure transducer (P23 ID Gould, Statham Instrument, Nottingham, UK) coupled to a computerized data acquisition system (PowerLab®, ADInstruments, Sydney, Australia). A midline abdominal incision was performed to expose the left kidney and the renal artery.

Upon completion of the animal surgical procedure and before commencing the experimental protocol, 2 mL of normal saline (i.v.) were given to the animal, after which it was left for a period of 1 h to stabilize (Armenia et al., 2004; Khan et al., 2007). At the end of the experiment, the animals were given an overdose of anesthesia (Sodium pentobarbitone, Nembutal®, CAVE, France) and disposed of in accordance with the guidelines of the Animal Ethics Committee of USM, Penang, Malaysia.

**Experimental protocol**

**Acute renal denervation (ARD):** ARD of the left kidney was carried out by stripping 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 then cut. This was followed by coating of the remaining covering tissue with a solution of 10% phenol in absolute alcohol (Bello-Reuss et al., 1975; Ogawa et al., 2002; Salman et al., 2008). The animal was then allowed to stabilize for an hour before commencing the clearance study. In the control innervated animals, the renal nerve was left intact and the animal was allowed to stand for an equivalent time period before starting urine and plasma samples collection.

**Validation of renal denervation procedure:** In a different set of experiments, functional loss of the renal sympathetic nerve action was confirmed by carrying out renal nerve stimulation (RNS). Twelve SD rats were randomly divided into two groups of 6 animals each. The first group was subjected to ARD, while in the second group the renal nerve was left intact to serve as a control subset. The loss of the functions of the renal nerves was tested by stimulating them (Grass S 48 stimulator, Grass instrument, MA, USA) at 15 V, 0.2 msec, 1-4 Hz for 1.5-30 sec in ascending and descending manners. Changes in renal blood flow (RBF) in response to electrical stimulation of the renal nerve were measured by placing a flowmeter probe (FP 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®, ADInstrument, Australia) (Salman et al., 2008).
Clearance study: In both denervated and innervated SD and SHR rats, the left ureter was cannulated (PE10, Portex, UK) and six urine clearances were collected at 20 min intervals for 2 h to measure urine volume and urine sodium ($U_{\text{Na}}$) and subsequently calculate the urine flow rate (UFR) and absolute sodium excretion ($U_{\text{Na}}V$). Plasma samples, on the other hand, were obtained at the same time intervals for the measurement of plasma sodium ($P_{\text{Na}}$) levels.

Analytical procedures

Biological samples and biochemical analysis: Blood samples were collected (0.5 mL) from the right carotid artery into a pre-cooled syringe, centrifuged (3000 rpm, 1 min) and the clear plasma was separated. The blood cells were re-suspended in normal saline at an equal volume to the plasma obtained and re-infused into the animal instantly (Abdul Sattar, 1994; Armenia, 2004). Plasma and urine samples were stored at -4°C until assayed for sodium using flame photometry.

Calculations: Calculations of the renal functional clearance parameters were done using the following expressions:

Urine flow rate (UFR): The volume of urine excreted per unit time which is termed as UFR was calculated by:

$$\text{UFR} (\mu L \text{ min}^{-1} \text{ kg}^{-1}) = \frac{\text{UV} (\mu L)}{T (\text{min}) \times \text{wt. (kg)}}$$

where, UV is the urine volume, T is the time and wt. is the weight of the animal.

Absolute sodium excretion ($U_{\text{Na}}V$): $U_{\text{Na}}V$ reflects the actual amount of sodium excretion in urine and is the result of multiplying urine flow rate by urine concentration of sodium:

$$U_{\text{Na}} (\mu \text{mol} \text{ min}^{-1} \text{ kg}^{-1}) = U_{\text{Na}} (\mu \text{mol} \text{ L}^{-1}) \times \text{UFR} (\mu L \text{ min}^{-1} \text{ kg}^{-1})$$

where, $U_{\text{Na}}$ is urine sodium level and UFR is urine flow rate.

Kidney index (KI): At the end of each experiment and when the animal was sacrificed, the contralateral kidney was collected. The collected kidney was cleared from any connective tissue, blotted on tissue paper and weighed.

Finally, the kidney index was calculated from the values of body and kidney weights using the equation:

Kidney index (%) = \frac{\text{kidney weight (g)}}{\text{animal weight (g)}} \times 100

Statistical analysis: All data were expressed in terms of mean±SEM and comparison of values between groups was done using one- and two-way analysis of variance (ANOVA) followed by Bonferroni/Dunnett (all mean) post hoc test (Superanova, Abacus Inc., Barkley, CA, USA). The differences between the means were considered significant at a probability value of less than 5%.

RESULTS

General observations: Body weight of SD and SHR rats following 1-4-16 h of fasting and their kidney index with or without ARD are shown in Table 1. The data show no significant differences among experimental groups.

Effect of RNS on RBF responses following ARD:

Blanching of the kidney in response to electrical stimulation, which is usually observed in intact renal nerves, was completely lost after ARD. It was further observed that the overall mean percentage reduction in the RBF was significantly attenuated in the denervated SD rats as compared to the rats with intact renal nerves (36.0±4.0 versus 12.7±2.6%, p<0.05) (Fig. 1).

![Reduction in RBF](image)

**Fig. 1:** Renal nerve stimulation (RNS) effect on % reduction in renal blood flow (RBF) in denervated (DNX) and innervated (INN) SD rats. Data presented as mean±SEM (n = 6). *p<0.05, denervated versus innervated SD rats. Data were analyzed by two-way ANOVA followed by Bonferroni/Dunnett post-hoc test
Effect on mean arterial blood pressure (MAP): There was no significant difference in MAP in denervated and innervated normotensive animals (111.9±2.9 versus 108.4±3.9 mmHg, p>0.05). Likewise, no significant difference in MAP values was seen in denervated and innervated hypertensive rats (144.3±2.1 versus 147.5±3.1 mmHg, p>0.05). However, significantly high MAP readings were observed in innervated SHR rats in comparison to innervated normotensive SD rats (111.9±2.9 versus 144.3±2.1 mmHg; p<0.05). The significant difference in MAP level was almost maintained even following denervation procedure in both animal species (108.4±1.9 versus 147±3.1 mmHg; p<0.05) (Fig. 2).

Effect of acute unilateral renal denervation on urine flow rate (UFR), absolute sodium excretion ($U_{\text{Na}} V$) and plasma sodium ($P_{\text{Na}}$) in SD and SHR rats: Present study demonstrates that ARD in SD and SHR rats contributes to a marked increase in the UFR measured at 20 min intervals for 2 h as compared with that measured at the same time points in innervated counterparts (90.7±8.5 versus 27.5±2.6 $\mu$L min$^{-1}$ kg$^{-1}$ and 136.9±9.8 versus 43.9±3.5 $\mu$L min$^{-1}$ kg$^{-1}$, respectively; p<0.05). UFR in SD and SHR, which remained constant in both cases before denervation (27.5±2.6 versus 43.9±3.5 $\mu$L min$^{-1}$ kg$^{-1}$; p<0.05), were considerably higher in SHR than SD rats after ARD (136.9±9.8 versus 90.7±8.5 $\mu$L min$^{-1}$ kg$^{-1}$; p<0.05) (Fig. 3).

Like UFR, it was further observed that $U_{\text{Na}} V$ levels, which were measured using the same renal clearance collection protocol were significantly higher in denervated SD and SHR rats as compared to those measured at the same time intervals in rats with intact renal nerves (13.7±1.5 versus 4.4±0.5 $\mu$mol min$^{-1}$ kg$^{-1}$ and 24.4±1.9 versus 7.5±0.6 $\mu$mol min$^{-1}$ kg$^{-1}$, respectively; p<0.05). Moreover, values of $U_{\text{Na}} V$ tended to remain unchanged in intact-renal-nerves SD and SHR rats (4.4±0.5 versus 7.5±0.6 $\mu$mol min$^{-1}$ kg$^{-1}$; p<0.05), though they were significantly elevated in denervated SHR rats as compared to their denervated normotensive counterparts (24.4±1.9 versus 13.7±1.5 $\mu$mol min$^{-1}$ kg$^{-1}$; p<0.05) (Fig. 4).
Fig. 5: Plasma sodium ($P_{Na}$) in denervated (DNX) and innervated (INN) SD and SHR rats. Data presented as mean±SEM (n = 6). *p<0.05, innervated SD versus innervated SHR rats. Data were analyzed by two-way ANOVA followed by Bonferroni/Dunnett post-hoc test.

$P_{Na}$ remained constant in both SD and SHR denervated and innervated cases (145.1±1.1 versus 137.7±2.3 mmol L$^{-1}$ and 139.1±2.3 versus 145.6±1.3 mmol L$^{-1}$, respectively; p>0.05). However, the innervated SHR rats demonstrated higher $P_{Na}$ levels as compared to the innervated normotensive ones (145.6±1.3 versus 137.7±2.3 mmol L$^{-1}$; p<0.05) with the sodium levels in plasma of denervated SHR rats tended to approximate the values in innervated SD rats (139.1±2.3 versus 137.7±2.3 mmol L$^{-1}$; p>0.05) (Fig. 5).

DISCUSSION

The kidney is predominantly and extensively permeated by adrenergic intrinsic nerves. Renal neural input is comprised of efferent and afferent innervations. The efferent sympathetic nerve fibers supply all the segments of renal vasculature, entering at the kidney hilus. These sympathetic nerve inputs are distributed throughout the renal cortex and outer strip of the medulla having the highest density in the juxtamedullary region of the inner cortex and the lowest in the renal tubules (Barajas et al., 1992; DiBona, 2000). These innervations follow the vasa recta into the outer medulla and concurrently vanish with the disappearance of smooth muscle cells (Dinerstein et al., 1979). The sensory afferent renal nerves, on the other hand, are primarily localized in the corticomedullary connective tissues of the pelvic region and major vessels (Barajas et al., 1992). The renal sympathetic innervation of the kidney greatly influences various aspects of renal functions, including renal hemodynamics, tubular sodium and water reabsorption and renin secretion. These effects provide an imperative control system which is central to the physiological regulation of arterial blood pressure, total body fluid and sodium homeostasis (DiBona, 1994; Salomonsson et al., 2000).

Abnormalities in the functional integrity of the renal sympathetic nerve regulatory mechanism result in major pathophysiological consequences and are evident in clinically relevant human disease states. Low RNSA causes an impaired renin secretion, failure to conserve sodium normally and an attenuated ability to get rid of both acute and chronic sodium loads. Conversely, high RNSA contributes considerably to excess renal retention of sodium and its related renal abnormalities seen in both hypertension and edema forming conditions, such as heart failure, cirrhosis and nephrotic syndrome (DiBona, 1994).

As the sympathoadrenal system plays an important role in the development of hypertension where increased sympathetic activity is present in different tissues (heart, vasculature and kidney) of SHR rats (Hall and Brands, 2000) and in an attempt to examine the importance of the renal sympathetic nerve in the regulation of renal functions and, hence, pathogenesis of hypertension, the main objectives of this study were to investigate the changes in systemic hemodynamics, salt and water excretion in SD and SHR rats in response to ARD along with the quantification of the renal salt and water excretory functions under normal and high blood pressure conditions where alterations in the renal sympathetic nerve action are thought to play a role.

In this study, the efficiency of the ARD procedure was assessed by the application of an electrical stimulus (15 V, 0.2 msec, 1-4 Hz, 15-30 sec) to the renal nerves before and after denervation. The data revealed a marked drop in RBF in response to RNS in animals with intact renal nerves. The latter finding can be attributed to the high sympathetic discharge released by the intact nerve endings upon stimulus application, an effect which was almost lost following renal denervation. Furthermore, blanching of the kidney in response to RNS, which is usually seen in the intact renal nerves, completely disappeared after ARD. These findings collectively confirmed the effectiveness, reliability and reproducibility of this technique in removing any possible influences of the sympathetic nerves on the kidney.

Regardless of the animal species, present data demonstrated that MAP was approximately the same in both denervated and innervated rats. Jacob and colleagues showed that in chronic renal denervation (CRD), several adaptive mechanisms, which are thought to play a contributory role in the blood-pressure-lowering effect following a denervation procedure, might become
involved, such as increased sodium and water excretion, suppression of the renin-angiotensin-aldosterone system (RAS) in response to the loss of renal sympathetic tone and/or reduction in the renal vasomotor tone which consequently results in an overall decrease in total peripheral vascular resistance (Jacob et al., 2003). These adaptive changes require a substantial period of time to develop and hence accompany CRD. Thus, the unchanged MAP in response to ARD in SD and SHR experimental animals according to present results signifies that the above adaptive changes will only come into play over a considerable period of time after denervation. The last suggestion may additionally explain why there was no significant change in the plasma levels of sodium in both denervated and innervated rats. However, the considerably higher MAP values in SHR rats, along with the hyperactive sympathetic tone, contributed to higher Psa levels in these animals as compared to the normotensive ones. Once ARD was performed, the sodium levels tended to approximate the values in SD rats regardless of any changes in the pressure values.

In this study, instantaneous measurable changes in the salt and water excretions were seen directly after renal denervation. There was approximately a three-fold increase in Uva and UFR in denervated animals as compared to their innervated equivalents, irrespective of the blood pressure differences between SD and SHR rats. These results suggest that renal sympathetic activity is involved in sodium and water regulation. The observed diuresis and natriuresis in response to ARD in these animals coincides with many earlier reported observations that typically depicted these changes following renal denervation (Bello-Reuss et al., 1975; Knox and Spielman, 1983; Salman et al., 2008). Furthermore, it provides support for the efficiency and accuracy of the renal denervation procedure used. Presented data showed that there is a strong correlation between the RSNA of the normotensive and hypertensive animals and the observed pattern of salt and water excretions following removal of neural input to the kidney. The latter fact was confirmed in that the urine flow and sodium excretion values were comparable in both SD and SHR rats with intact renal nerves, but once the nerve functions were stopped by ARD, these values became significantly different, indicating the highly exaggerated activity of the renal sympathetic nerves in the hypertensive animals, a finding which has supported a stated view that a disturbance of the blood pressure-natriuresis relationship is responsible for elevated blood pressure (Ritz et al., 2003).

In conclusion, this study reaffirmed the significant contribution of renal nerves to the regulation and control of renal hemodynamics and functions. Diuresis and natriuresis following ARD are well-depicted and reproducible phenomena and are dependent on the exclusion of neural stimulatory influences regardless of variations in basal MAP values in normotensive and hypertensive animals. The extent of the increase in sodium and water excretions is approximately comparable after ARD in SD and SHR rats; however, enhanced salt and water excretions following ARD are more profound in hypertensive animals, which emphasizes the high renal sympathetic nerve discharge in these animals and highlights the significant contribution of renal nerves to the genetic model of essential hypertension.

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