Role of norepinephrine & angiotensin II in the neural control of renal sodium & water handling in spontaneously hypertensive rats


Department of Cardiovascular & Renal Physiology & Pharmacology, School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang, *Department of Pharmacology, Faculty of Medicine, Universiti Malaya, Kuala Lumpur, Malaysia, **College of Pharmacy, University of Baghdad, Baghdad, Iraq, + Department of Physiology, Tulane Hypertension & Renal Center of Excellence, Tulane University Health Sciences Center, New Orleans, Louisiana, USA & #Department of Physiology, University College Cork, Cork, Ireland

Received February 24, 2009

Background & objectives: A wealth of information concerning the essential role of renal sympathetic nerve activity (RSNA) in the regulation of renal function and mean arterial blood pressure homeostasis has been established. However, many important parameters with which RSNA interacts are yet to be explicitly characterized. Therefore, the present study aimed to investigate the impact of acute renal denervation (ARD) on sodium and water excretory responses to intravenous (iv) infusions of either norepinephrine (NE) or angiotensin II (Ang II) in anaesthetized spontaneously hypertensive rats (SHR).

Methods: Anaesthetized SHR were acutely denervated and a continuous iv infusion of NE (200 ng/min/kg) or Ang II (50 ng/min/kg) was instigated for 1 h. Three 20-min urine clearances were subsequently collected to measure urine flow rate (UV) and absolute sodium excretion (U\textsubscript{Na}V).

Results: Higher UV and U\textsubscript{Na}V (P<0.05) were observed in denervated control SHR as compared to innervated counterparts. The administration of NE or Ang II to innervated SHR produced lower UV and U\textsubscript{Na}V (P<0.05 vs. innervated control SHR). Lower diuresis/natriuresis response to ARD was observed in NE-treated SHR compared to denervated control SHR (P<0.05). Salt and water excretions in denervated NE-treated SHR, however, were significantly higher (P<0.05) relative to the excretion levels in control denervated SHR. Conversely, there was a higher (all P<0.05) diuresis/natriuresis response to ARD when Ang II was administered to SHR compared to denervated control or innervated Ang II-treated SHR.

Interpretation & conclusions: NE retains its characteristic antidiuretic/antinatriuretic action following ARD in SHR. Typical action of Ang II on salt and water excretions necessitates the presence of an intact renal innervation. Ang II is likely to facilitate the release of NE from renal sympathetic nerve terminals through a presynaptic site of action. Moreover, there is a lack of an immediate enhancement in the renal sensitivity to the actions of NE and Ang II following ARD in a rat model of essential hypertension.

Key words: Acute renal denervation - angiotensin II - diuresis - natriuresis - norepinephrine - spontaneously hypertensive rats
An imperative role for renal sympathetic nerve activity (RSNA) has been suggested in the maintenance of renal function and mean arterial pressure (MAP)\(^1,2\). Renal sympathetic nerves innervate the tubules, the vessels, and the juxtaglomerular granular cells of the kidney\(^1\). In this way, alterations in RSNA directly influence the function of these innervated renal effector units. Increases in RSNA decrease urinary sodium and water excretion by increasing renal tubular water and sodium reabsorption throughout the nephron, decrease renal blood flow (RBF) and glomerular filtration rate (GFR) by constricting the renal vasculature, and increase the activity of the renin-angiotensin system (RAS)\(^3,4\). On the contrary, removal of the neural input by renal denervation leads to inhibition of tubular transport (denervation diuresis and natriuresis)\(^5,6\) and, under some circumstances, to a decrease in renin secretion and angiotensin II (Ang II) formation\(^7\).

It has been suggested that the effect of RSNA on arterial pressure and intravascular volume is, at least in part, mediated through the regulation of RAS and basal Ang II levels\(^2\). It has further been shown that the effect of Ang II to increase proximal tubular chloride and water reabsorption decreases by about 75 per cent following renal denervation and only a very small portion of the Ang II effect (~25%) can be ascribed to a direct action on Ang II receptors located on proximal tubules\(^4,8\). Based on these findings, it has been proposed that Ang II in the kidney is likely to facilitate the release of norepinephrine (NE) from renal sympathetic nerve terminals through a presynaptic site of action\(^3,8\). Yet, how these endogenously present neurohormonal mediators perform their characteristic mechanistic action to influence renal salt and water excretions under a pathophysiological state mainly characterized by sustained activation of the renal sympathetic nerves such as essential hypertension is still unclear.

For decades, discerning the role of the renal adrenergic neurons in the regulation of renal function has been popularly achieved through renal denervation\(^9-11\). One concern with this approach is that increased renal sensitivity to NE may develop. This may in turn decrease the apparent magnitude of the effect of the renal nerves or indeed totally mask their action\(^11\). It has been shown that chronically denervated kidney in the presence of ganglionically blocked contralateral kidney shows greater decreases in RBF, GFR and sodium excretion in response to NE infusion\(^12\). Lohmeier and co-workers observed no significant differences in sodium excretions between innervated and chronically denervated kidneys in response to infusions of NE at physiologically appropriate levels\(^10\). Therefore, the issue also arises as whether, under a pathophysiological condition of genetic hypertension, there may be an abrupt increase in the renal responsiveness to NE and Ang II following acute removal of renal sympathetic nerve action.

With this background, the aim of the present study was to characterize the interrelationship between renal sympathetic nerves and RAS and their contribution to the regulation of renal sodium and water handling under conditions of enhanced RSNA in spontaneously hypertensive rats (SHR). This issue was addressed through evaluating the changes in salt and water excretion in response to the use of exogenously administered intravenous (iv) infusions of NE and Ang II following acute unilateral renal denervation. This study has further investigated whether acute renal denervation (ARD) could lead to enhanced renal sensitivity to NE and Ang II.

**Material & Methods**

*Animals and experimental groups:* Adult male SHR (250-300 g) obtained from the Animal Care Facility of Universiti Sains Malaysia (USM), Penang, Malaysia were used in this study. The animals were housed in standard plastic cages with 12:12 h light-dark cycle and fed with normal commercial rat chow (Gold Coin Feed Mills Sdn Bhd, Malaysia) and water ad libitum. Animal handling and all procedures on animals were carried out in accordance with the guidelines of the Animal Ethics Committee, USM. After acclimatization of a week, animals were randomly divided into 6 groups of 6 each. Group (1) control innervated SHR, group (2) control denervated SHR, group (3) innervated NE-treated SHR, group (4) denervated NE-treated SHR, group (5) innervated Ang II-treated SHR and group (6) denervated Ang II-treated SHR.

*Drugs and chemicals:* NE and Ang II were purchased from Sigma Chemical Co., USA and stock solutions were prepared by dissolving in isotonic saline (0.9% NaCl solution). Phenol was purchased from Sigma Aldrich (Chemic GmbH, Germany) and a 10 per cent solution was obtained by dissolving in absolute alcohol (R & M Chemicals, Essex, UK).

*Surgical preparation of animal:* The rats were fasted overnight with unlimited access to drinking water. Anaesthesia was induced by sodium pentobarbitone (Nembutal®; CAVE, France) at a dose of 60 mg/kg (ip). After a tracheotomy (PE250, Portex, UK), a
polyethylene catheter (PE50, Portex, UK) was inserted into the left jugular vein to enable the administration of an iv maintenance infusion of isotonic saline (0.9 % NaCl) at an infusion rate of 6 ml/h and to allow an intermittent administration of supplementary bolus injections of the anaesthetic (10 mg/kg in normal saline). The right carotid artery was similarly catheterized (PE50, Portex, UK) for blood samples collection and 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 made to expose the left kidney. The left ureter was cannulated (PE10, Portex, UK) for collection of urine samples.

Upon completion of the surgical procedure, 2 ml of saline (iv) were given to the animal, after which it was stabilized for 1 h. 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.

Acute renal denervation (ARD): As described in our earlier studies, renal denervation of the left kidney was done by stripping and cutting the nervous and connective tissue around the left renal arteries and veins and coating the renal artery with 10 per cent phenol in absolute ethanol. In innervated animals, renal nerves were left intact.

Clearance study:

Control innervated SHR group - The animals were maintained on an iv infusion of saline and subsequently, three 20-min urine samples were collected.

Control denervated SHR group - The animals were maintained on an iv infusion of saline. Renal denervation was done (≈15 min) and thereafter three 20-min urine samples were collected.

Innervated NE- or Ang II-treated SHR groups - The iv maintenance infusion of normal saline was replaced by either NE (200 ng/min/kg) or Ang II (50 ng/min/kg) infusions. The choice of the doses was based upon a pilot study in SHR to determine suitable doses that would ultimately result in an increase in MAP of no more than 10-15 mmHg above its basal value. Following infusion of these drugs, three 20-min urine samples were collected.

Denervated NE- or Ang II-treated SHR groups - Initially, the animals were maintained on an iv infusion of saline. Following renal denervation the iv maintenance infusion of normal saline was replaced by either NE (200 ng/min/kg) or Ang II (50 ng/min/kg) infusions after which three 20-min urine samples were collected.

Analytical procedures:

Biological samples and biochemical analyses - Urine samples were collected in microcentrifuge tubes (Eppendorf, Hamburg, Germany) and the volumes obtained were gravimetrically quantified. Urine samples were stored at -4 °C until assayed for sodium using flame photometry (Hitachi, Japan).

Calculations - Urine flow rate was calculated by the following formula: UV (µl/min/kg) = V (µl) / T (min) x BW (kg). 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.

Absolute excretion of sodium was calculated using the equation: U_{Na}V (µmol/min/kg) = U_{Na} (µmol/µl) x UV (µl/min/kg). Here, U_{Na}V is the absolute urinary excretion of sodium and U_{Na} is the urine concentration of sodium.

Statistical analyses: 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 (SuperANOVA, Abacus Inc., Barkley, CA, USA). The differences between the means were considered significant at 5 per cent level.

Results

Basal MAP values in experimental groups: MAP (mmHg) basal values were 147.4 ± 1.9, 143.1 ± 2.9, 155.4 ± 4.0, 157.4 ± 4.0, 158.9 ± 8.3 and 155.9 ± 8.3 for control innervated, control denervated, innervated NE-treated, denervated NE-treated, innervated Ang II-treated and denervated Ang II-treated SHR rats, respectively.

Changes in salt and water excretions: It was observed that the induction of ARD in the left kidney contributed to significantly higher UV and U_{Na}V (P<0.05 vs. innervated control SHR). In contrast, lower (P<0.05) UV and U_{Na}V were observed upon the administration of NE or Ang II to innervated SHR as compared to innervated rats administered the vehicle only. NE infusion in denervated animals produced a significantly lower denervation-induced diuresis and natriuresis (P<0.05 vs. denervated control SHR). These
responses, however, remained higher ($P<0.05$) relative to innervated NE-treated SHR rats. Conversely, Ang II infusion in denervated SHR produced a significantly higher (all $P<0.05$) diuretic/natriuretic response as compared to innervated Ang II-treated or denervated vehicle-treated SHR (Fig. 1).

**Fig. 1.** Effect of norepinephrine (NE) and angiotensin II (Ang II) infusions on (A) urine flow rate (UV) and (B) absolute sodium excretion ($U_{\text{Na}}V$) following acute renal denervation (ARD) in spontaneously hypertensive rats (SHR). Data presented as mean ± S.E.M. ($n=6$). $^a$ indicates $P<0.05$, innervated norepinephrine-treated SHR (INN + NE) vs. control innervated SHR (INN). $^b$ indicates $P<0.05$, innervated angiotensin II- treated SHR (INN + Ang II) vs. control innervated SHR (INN). $^c$ indicates $P<0.05$, control innervated SHR (INN) vs. control denervated SHR (DNX). $^d$ indicates $P<0.05$, denervated norepinephrine-treated SHR (DNX + NE) vs. control denervated SHR (DNX). $^e$ indicates $P<0.05$, denervated norepinephrine-treated SHR (DNX + NE) vs. innervated norepinephrine-treated SHR (INN + NE). $^f$ indicates $P<0.05$, denervated angiotensin II- treated SHR rats (DNX + Ang II) vs. control denervated SHR (DNX). $^g$ indicates $P<0.05$, denervated angiotensin II- treated SHR rats (DNX + Ang II) vs. innervated angiotensin II- treated SHR (INN + NE). Data were analyzed by two-way ANOVA followed by Bonferonni-Dunnett (all mean) post-hoc test.

**Pressure-diuresis (P/D) and pressure natriuresis (P/N) responses:** In the absence of any significant changes in MAP before and following ARD, significantly ($P<0.05$) higher UV and $U_{\text{Na}}V$ mean responses were observed compared to control innervated SHR (INN) in both groups (Fig. 2).

**Fig. 2.** (A) Pressure-diuresis (P/D) and (B) Pressure-natriuresis (P/N) responses to norepinephrine (NE) and angiotensin II (Ang II) infusions following acute renal denervation (ARD) in spontaneously hypertensive rats (SHR). Data presented as mean ± S.E.M. ($n=6$). $^a$ indicates $P<0.05$, innervated norepinephrine-treated SHR (INN + NE) vs. control innervated SHR (INN). $^b$ indicates $P<0.05$, innervated angiotensin II-treated SHR (INN + Ang II) vs. control innervated SHR (INN). $^c$ indicates $P<0.05$, control innervated SHR (INN) vs. control denervated SHR (DNX). $^d$ indicates $P<0.05$, denervated norepinephrine-treated SHR (DNX + NE) vs. control denervated SHR (DNX). $^e$ indicates $P<0.05$, denervated norepinephrine-treated SHR (DNX + NE) vs. innervated norepinephrine-treated SHR (INN + NE). $^f$ indicates $P<0.05$, denervated angiotensin II-treated SHR rats (DNX + Ang II) vs. control denervated SHR (DNX). $^g$ indicates $P<0.05$, denervated angiotensin II-treated SHR rats (DNX + Ang II) vs. innervated angiotensin II-treated SHR (INN + NE). Data were analyzed by two-way ANOVA followed by Bonferonni-Dunnett (all mean) post-hoc test.
seen in response to renal denervation in SHR compared to innervated littermates. The shift in the baseline of MAP following NE infusion was associated with lower \((P<0.05)\) mean values of UV and \(U_{Na}V\) in both denervated and innervated animals. On the contrary, upon infusing Ang II to the animals the shift in the baseline of MAP was accompanied by significantly \((P<0.05)\) lower mean values of UV and \(U_{Na}V\) in innervated SHR rats, but significantly higher levels of these response variables were observed in denervated counterparts (Fig. 2).

**Discussion**

In the present study, we attempted to assess the relative contribution of neurally-mediated Ang II release versus the direct effects of sympathetic activity on renal sodium and water handling in a rat model of essential hypertension in which augmented RSNA is a constant feature. Herein, we provide evidence that exogenous supplementation of NE following mechanico-chemical renal denervation in SHR retains characteristic antidiuretic and antinatriuretic effects. On the other hand, the ability of Ang II to significantly lower salt and water excretion seems to be lost or even reversed upon its administration to denervated SHR. We further showed that renal hyper-responsiveness to the actions of NE and Ang II is totally absent shortly following the loss of sympathetic nerve function in rats with a genetic model of hypertension.

In our study SHR strain was chosen since increased central sympathetic discharge to various organs has been well demonstrated in this animal model of essential hypertension\(^{14-16}\). Despite the fact that the cause of the sympathetic hyperactivity is not well understood, it is possible that a general increase in excitability of elements within the central nervous system of SHR that regulates sympathetic activity is likely to be a main cause. In comparison to Wistar-Kyoto (WKY) rats, a normotensive genetic control strain, the hyper-responsiveness in SHR is suggested by the findings that larger increases in plasma catecholamine levels and sympathetic nervous activity have been reported\(^{17,18}\). Therefore, a hypothesis was formulated that somato-sympathetic reflexes and responses to vasoactive stimuli might be enhanced in these animals rendering them a useful research tool to test our objectives.

To rationalize the choice of the doses of NE and Ang II used in our experiments, P/D and P/N responses were plotted. These plots are usually constructed under instances where increased MAP is seen. The use of P/D and P/N graphs was of great importance to evaluate the changes in salt and water excretion from denervated kidneys subjected to the action of a vasoconstricting substance which could contribute to an elevated MAP. Generally, high cardiovascular pressure causes the baroreceptors to enhance their rate of firing to transmit more impulses via the afferent neurons and the descending pathways to the hypothalamus resulting in a reduction in vasopressin secretion which may ultimately contribute to enhanced diuretic and natriuretic responses to normalize the elevated blood pressure\(^{19}\). In our study, we used vasoconstrictor doses which would ultimately result in no more than 10-15 mmHg increase in MAP, a pressure change which does not exceed the autoregulatory range\(^{20,21}\). In this context, P/D and P/N plots showed that the significant changes in salt and water excretions appeared to take place within a relatively small range of pressure values. Thus, it is unlikely that the observed changes in salt and water excretions were due to effects other than the direct renal action of NE and Ang II since the shift in MAP following NE and Ang II administration was associated with typical antidiuretic and antinatriuretic responses rather than pressure diuresis and natriuresis.

We observed instant elevations in salt and water excretions after renal denervation in SHR which are in agreement with our earlier reports on sodium and water regulation following removal of renal sympathetic tone in hypertensive animals\(^{5,13}\). Further, the effectiveness of this procedure in eliminating the action of renal sympathetic outflow has already been verified in a previous study from our laboratory\(^5\).

Our data demonstrated lower diuresis and natriuresis responses to ARD following the administration of NE infusion. Normally, NE released by the sympathetic nerve endings causes salt and water reabsorption through a direct action on the renal tubules, reduction in RBF via activation of postsynaptic \(\alpha_2\)-adrenergic receptors and to a lesser extent postsynaptic \(\alpha_2\)-adrenergic receptors on the renal vasculature, and/or a direct action on \(\beta_2\)-adrenergic receptors on the juxtaglomerular cells which consequently augments renin secretion and Ang II formation\(^{3,4,20,22,23}\). By renal denervation, the endogenous NE released in response to stimulation of the renal sympathetic nerves is practically diminished. Therefore, exogenous IV supplementation of NE to denervated SHR appeared to produce similar renal functional responses to the endogenous one released by an intact renal innervation. Interestingly, the antidiuretic/antinatriuretic action
of Ang II was distinctly reversed in SHR subjected to renal denervation. These results suggested that an intact renal innervation is a prerequisite for typical action of Ang II on salt and water excretion. Thus, a possible interaction between the renal sympathetic nervous system and RAS to control renal sodium and water handling in SHR can unswervingly be inferred. Together, our findings in SHR supported an earlier stated view that Ang II in the kidney is likely to assist the release of NE from renal sympathetic nerve terminals through a presynaptic site of action\textsuperscript{1,2}. However, the reason for the exaggerated diuretic/natriuretic action in response to Ang II in denervated SHR remains unclear and warrants further investigation.

Previously, it has been shown that chronic renal denervation results in increased expression of α-adrenoceptors (α-adrenoceptors upregulation) in the rat kidney and therefore contributes to higher responsiveness to the action of NE\textsuperscript{5,24}. Blockade of α-receptors has also been shown to blunt the increased responses to NE\textsuperscript{25}. Similarly, chronic renal denervation was found to increase Ang II receptors in the glomeruli of the denervated kidney\textsuperscript{36}. Thus, we hypothesized that these effects might become more prominent using a rat model of genetic hypertension in which enhanced sympathetic activity is well established\textsuperscript{5,13}. However, increased sensitivity to infusions of NE and Ang II following renal denervation was not observed in our experiments since the extent of salt and water excretion was maintained at significantly higher levels as compared to those of innervated rats administered either NE or Ang II. These findings suggested that renal hyper-responsiveness to catecholamine and Ang II is not an immediate consequence to ARD in SHR. The importance of this finding lies in the view that there might be a growing concern from increased sensitivity to the action of NE or Ang II if ARD is a method used in studies involving these vasoconstricting stimuli.

It is practically noteworthy to highlight the fact that several previous studies from our laboratory showed reproducible responses to NE and Ang II in surgically stressed rats\textsuperscript{5,6,27,28}. Thus it is unlikely that the measured renal functional responses to vesopresser agents did not retain sufficient level of accuracy when performed in anaesthetized rat model.

In summary, exogenously administered NE retains its antiuretic/antinatriuretic action following acute removal of renal sympathetic nerve action. Typical action of Ang II on tubular salt and water excretion necessitates the presence of an intact renal innervation indicating a likely interaction between the renal sympathetic nervous system and RAS to control renal sodium and water excretion in SHR, possibly via Ang II-induced presynaptic facilitation of NE release. ARD in SHR does not produce an immediate renal hyper-responsiveness to the action of NE and Ang II.

Acknowledgment

Authors acknowledge the Institute of Graduate Studies (IPS), Universiti Sains Malaysia, Penang, Malaysia, for financial support.

Conflicts of interest: No competing financial interests exist.

References


Reprint requests: Dr Ibrahim M. Salman, Department of Cardiovascular & Renal Physiology & Pharmacology
School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia
e-mail: ibraheem_muhammed@yahoo.com