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THE INTERACTION BETWEEN RENIN-ANGIOTENSIN AND SYMPATHETIC SYSTEMS IN THE RENAL VASCULATURE OF WISTAR-KYOTO RATS

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The various interactions between the renin-angiotensin system and the sympathetic system have been established at different levels and have been shown to bear prominent pathophysiological implications. This study was undertaken to characterize the renal responses to acute unilateral renal denervation of the left kidney in anaesthetized Wistar-Kyoto rats (WKY) by examining the effect of acute unilateral renal denervation on the renal hemodynamic responses to renal nerve stimulation (RNS). Twenty-four male WKY rats underwent acute unilateral renal denervation. After 7 days treatment with losartan, the overnight fasted rats were anesthetized (sodium pentobarbitone, 60 mg/kg i.p.) and renal vasoconstrictor experiments were done. The renal nerves were directly stimulated at a sequence of frequencies of 1, 2, 4, 6, 8, and 10 Hz at 0.2 ms duration and 15V for a period of 15 seconds in ascending and descending manner. The changes in the renal vasoconstrictor responses were determined in terms of reductions in renal blood flow caused by renal nerve stimulation. The data showed that there was significantly (all $P<0.05$) decreased renal vascular responsiveness to neural stimuli in denervated rats compared to those with intact renal nerves. In losartan treated denervated WKY rats, there were significant (all $P<0.05$) reductions in the renal vasoconstrictor responses to neural stimuli as compared to that of untreated denervated WKY rats. These data also suggested a possible interaction between sympathetic nervous system (SNS) and renin-angiotensin system (RAS) in terms of a crosstalk relationship between renal AT_1 and α_1-adrenoceptor subtypes in the renal vasculature of normal rats.

Keywords : α_1-adrenoceptors; renal hemodynamics; spontaneously hypertensive rat; losartan.

INTRODUCTION

The kidneys are endowed with rich innervations of sympathetic nerves extending to the vasculature and tubules. Indeed, the renal sympathetic nerves are increasingly considered as being important in regulating renal hemodynamic and thus blood pressure[1].

Apart from an important regulatory influence, systemic blood pressure and intravascular volume regulations are also significantly modified by the actions of reninangiotensin system (RAS) in the kidney. Circulating angiotensin II itself then interacts with the SNS at various sites and appears to amplify sympathetic activity. It may act on the brain to increase sympathetic outflow, on the sympathetic ganglia and adrenal medulla to increase catecholamine release, and at presynaptic sympathetic nerve endings to facilitate sympathetic neurotransmission through an enhanced norepinephrine release[2, 3] and this will assist the sympathetic influence on the heart and the systemic circulation.[4]

There is a growing concern on the role of renal nerves in the regulation of renal functions and hemodynamics. The renal circulation, tubular reabsorption and release of renin are under multiple controls by the renal nerves, hormones and paracrine active agents.[5]

The interaction between noradrenaline and angiotensin II is particularly relevant as there are several chances for positive feedback between the two systems. Indeed, angiotensin II facilitates the release of noradrenaline from post-ganglionic noradrenergic nerves via AT_1 receptors.[6-7]

With this background, this study was aimed to study the contribution of renal sympathetic nerves and RAS in the regulation of renal hemodynamics, in an attempt to investigate if there is any interaction between SNS and RAS in normal rats. With this aim, studies were carried out by studying renal vasoconstrictions induced by neural stimuli in acutely denervated rats either treated or untreated with losartan. Losartan was used in line of the objective of this study to examine the interaction of SNS and RAS in a situation where AT_1 receptors at which the Ang II mainly acts in the vasculature are blocked.

METHODS

Animals

Male WKY rats weighing 250- 300 g were procured from the Animal Care Facility, Universiti Sains Malaysia, Penang, Malaysia. Animal handling and all procedures on animals were carried out in accordance with the guidelines of the Animal Ethics Committee, Universiti Sains Malaysia, Penang, Malaysia and had their approval. After acclimatization of a week, animals were randomly divided into 3 groups namely control, losartan treated and sympathectomised losartan treated groups (all n = 16).

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Rats of the treated group received losartan for 7d at an oral dose of 10mg/kg/day as described earlier.[8-10]

Hemodynamic study

Surgical procedure

The overnight (10-12h) fasted rats were anaesthetized with 60 mg/kg (i.p.) sodium pentobarbitone (Nembutal®, CAVE, France). Following tracheostomy (PP250, Protex, UK) The right carotid artery was cannulated (PP50 Protex, UK) and connected to a pressure transducer (P23 ID Gould, Statham Instrument, Nottingham, UK) linked to a computerized data acquisition system (PowerLabâ, ADInstrumentation, Sydney, Australia) for the continuous monitoring of mean arterial blood pressure. The left jugular vein was cannulated to infuse maintenance doses of anesthetic whenever required. The left kidney was exposed via a ventral mid-line incision. The renal artery cleared to allow fitting of an electromagnetic flow probe (EP 100 series, Carolina Medical Instrument, King, North Carolina, USA). 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 (PowerLabÒ, ADInstrument, Australia). Upon completion of the surgery, 2 ml of normal saline (i.v.) was given to the animal and stabilized for an hour before commencing to the acute renal vasoconstrictor experiment.[11-13] At the end of the experiment, the animals were euthanized by an overdose of anaesthesia (Sodium pentobarbitone, Nembutals, CAVE, France) and disposed off in accordance with the guidelines of the Animal Ethics Committee of Universiti Sains Malaysia, Penang, Malaysia.

Acute renal denervation

Acute renal denervation of the left kidney was performed by stripping the renal artery out of its adventitia. All visible renal nerves passing from the celiac and aortico-renal ganglia to the kidney were isolated, dissected and then cut. This was followed by coating of the remaining covering tissue with a solution of 10% phenol in absolute alcohol as described previously.[14] The loss of the functions of the renal nerves was tested by stimulating (Grass S 48 stimulator, Grass instrument, MA, USA) them at 15V, 0.2ms, 10Hz for 15-30 seconds. Blanching of the kidney in response to electrical stimulation, which is usually observed in the intact renal nerves, was lost after renal

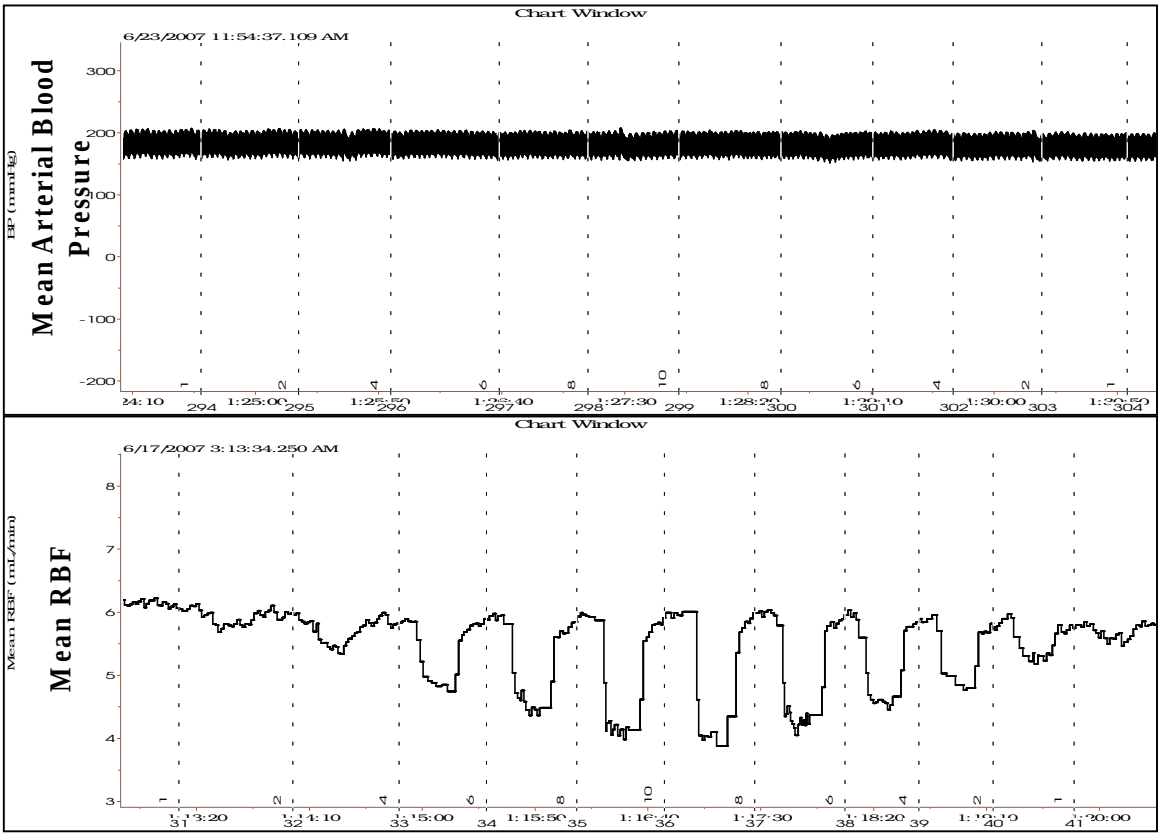


FIGURE - 1 Mean Arterial blood pressure and renal vasoconstrictor responses to RNS

denervation.

Renal vasoconstrictor experiments

Experimental procedure

Acute renal vasoconstrictor responses involved the application of direct electrical stimulation using (Grass S 48 Stimulator, Grass Instruments, MA, USA) on the renal nerves and that led to constriction of the renal vasculature, hence reduction in the renal blood flow (**Fig 1**). The renal nerves were directly stimulated at a sequence of frequencies of 1, 2, 4, 6, 8, and 10 Hz at 0.2 ms duration and 15V for a period of 15 seconds in ascending and descending manner.

Statistical analysis and presentation of data

The overall mean response for each frequency was taken as the average value of vasoconstrictor responses (drop in renal blood flow) obtained at each frequency. The data on the drop of renal blood flow were expressed as percentage drop of the renal blood flow in relation to the basal values of renal blood flow calculated at the beginning of the administration of the stimulus (renal nerve stimulation) used. All data were expressed as mean % reduction ± S.E.M. of renal vasoconstrictor responses elicited by all the frequencies and compared between control, losartan pre-treated and sympathectomised losartan pretreated rats. In the renal vasoconstriction experiments the statistical analysis of the data was done by two-way ANOVA and followed by the Bonferroni post hoc test using the statistical package Superanova (Abacus Inc., Barkley, CA, USA). The differences between the means were considered significant at 5% level.

RESULTS

Baseline values of Mean Arterial Pressure (MAP) and Renal Blood Flow (RBF)

There was no difference in the MAP of the acutely denervated rats and the rats with intact renal nerves i.e. the basal value of MAP in control rats was 110.28 ± 3.25, which is not significantly different ($p>0.05$) from sympathectomised rats baseline value 109.00 ± 7.20. Losartan did not affect the MAP i.e. the baseline MAP value for losartan treated sympathectomised rats 109.32 ± 8.89 was not significantly different ($p>0.05$) from the control 110.28 ± 3.25.

The basal values of renal blood flow showed no significant differences between sympathectomised and intact renal nerves treated and untreated rats. The baseline value of RBF in sympathectomised, sympathectomised losartan treated rats was not significantly different ($p>0.05$) from the control value i.e. (10.07 ± 2.57 and 11.81 ± 1.48 vs. 7.96 ± 1.03) respectively.

RENAL VASOCONSTRICTOR RESPONSES

Renal nerve stimulation

In both experimental groups of rats there were frequency-dependent decreases in RBF caused by direct electrical stimulation of the renal nerves **Fig. 2**. It was observed that the overall mean percentage decrease in the renal blood

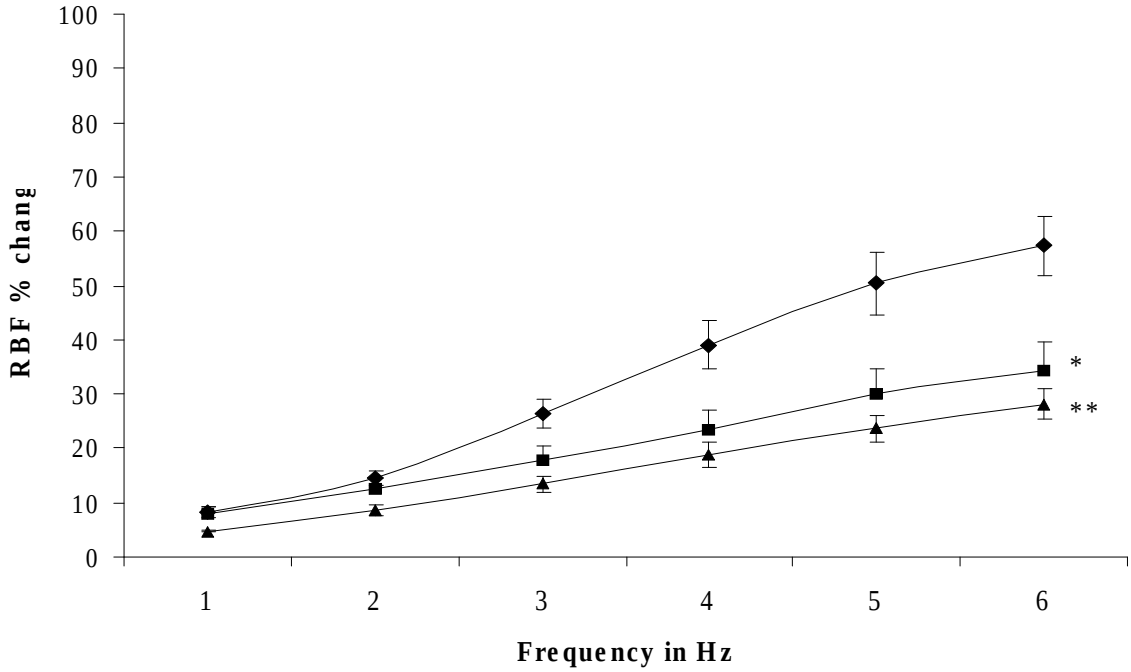
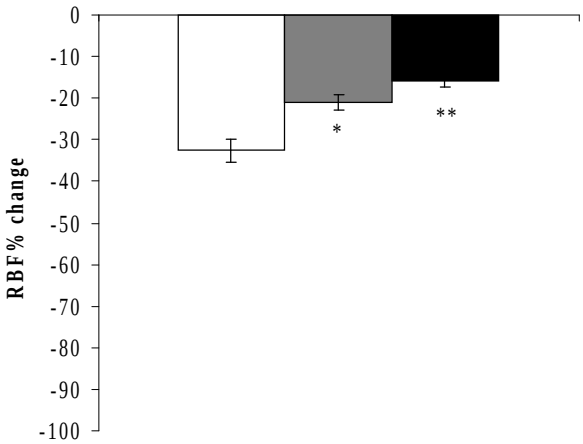


FIGURE - 2 Frequency- response curve showing the renal vasoconstrictor responses to RNS in the study groups

flow was significantly lower (all P<0.05) in the sympathectomised rats as compared to that of control (21.00 ± 1.78 vs. 32.64 ± 2.64) (**Fig. 3**). Moreover, there is significant attenuation (P<0.05) in the renal vasoconstrictor response to renal nerve stimulation after sympathectomy in losartan pretreated rats as compared to normal i.e. (15.86±1.37 vs. 32.64±2.64) and also if compared to sympathectomised control (21.00 ± 1.78) as shown in Fig. 3. Collectively, these results showed a significant decrease in the neurally induced renal vasoconstrictions in sympathectomised WKY rats treated with losartan as compared to control.



Overall % reduction in RBF in response to RNS

DISCUSSION

The kidney is richly supplied with nerves derived mainly from the celiac plexus and from the thoracic and lumber splanchnic nerves. The renal sympathetic nerves play important role in the regulation of renin release, tubular sodium and water reabsorption, and renal vascular resistance[1]. An increasing number of studies suggested important role of renal sympathetic nerves in the maintenance of renal functions and arterial blood pressure.[7, 15-16] It is also suggested that increase in the renal sympathetic tone increases vasoconstrictions by enhancing the activity of renin-angiotensin system (RAS).

The interaction between the renin-angiotensin system and the sympathetic nervous system has traditionally been regarded to be bidirectional. The α_1-adrenoceptors and AT_1 receptor subtypes play important role in the regulation of renal hemodynamic at the level of renal resistance vasculature. This study demonstrates that the vasoconstrictor response to renal nerve stimulation is reduced by AT_1 blockade. In our set of experiments it is feasible that the consequence of AT_1 blockade may reflect a desensitization of α_1-mediated renal vasoconstriction response that is well in accord with previous reports.[17] Ang II is known to facilitate the release of endogenous noradrenaline by activation of presynaptic AT_1 receptors. A study on pithed spontaneously hypertensive rats examined the sympatho-inhibitory actions of AT_1 receptor blocker irbesartan, showed that Ang II can enhance sympathetic neurotransmission by acting on AT_1 receptors that are located on sympathetic nerve terminals.[18]

The dose of the losartan used was indeed adequate to block the AT_1 receptors, and hence in providing an effective blockade on the action of Ang II in the renal vasculature of the WKY rats. Moreover this dose of losartan was used in several earlier studies.[9]

This study further suggested that there could be an enhanced sensitivity of the α_1-adrenoceptors to endogenous noradrenaline produced by neural stimulation. Whereby, it was observed that in the case of electrical stimulation of the renal nerves, the renal vasoconstrictions that were measured in terms of reductions in renal blood flow were markedly higher in the rats with intact renal nerve than that in the rats with renal sympathectomy. This perhaps was due to the destruction of the renal nerves followed by reduction of catecholamines level in the renal vasculature in the sympathectomized rats. A further reduction in the sympathectomized rats treated with losartan was probably due to the impact of both renal denervation and blockade of the action of Ang II in these rats.

In conclusion, this study showed that, the renal nerves play an important role in the neurally induced renal vasoconstrictions in WKY rats and there was an interesting crosstalk relationship between AT_1 and α_1-adrenoceptors in modulating the renal hemodynamics of acutely and unilaterally denervated rats. This study also showed that acute denervation of the kidney of the WKY rats is, indeed, possible by surgical procedure followed by a brief chemical insult to the renal nerves.

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REFERENCES

1. DiBona GF, Kopp UC. Neural control of renal function. Physiol Rev 1997; 77:75-197.

2. Walter R, Peter D, Andreas Z, Andreas D. Reduction of vascular noradrenaline sensitivity by AT1 antagonists depends on functional sympathetic innervation. Hypertension 2004; 44:346-351.

3. Reid IA. Interactions between ANG II, sympathetic nervous system, and baroreceptor reflexes in regulation of blood pressure. Am J Physiol 1992; 262: E763–778.

4. De Jonge AJ, Thoolen MJMC, Timmermans PBMWM, van Zwieten PA. Interaction of converting enzyme inhibition of converting enzyme inhibitors with the sympathetic nervous system. Progr Pharmacol 1984; 5: 25–38.

5. Grassi G. *J.* Renin-angiotensin-sympathetic crosstalks in hypertension: reappraising the relevance of peripheral interactions. Hypertens 2001; 19: 1713 -1716.

6. Elzbieta K-J, Agnieszka W, EJ. Johns. Early effects of renal denervation in the anaesthetized rat: natriuresis and increased cortical blood flow. J Physiol 2001; 531:527-534.

7. Squire IB, Reid JL. Interactions between the renin-angiotensin system and autonomic nervous system. In *The renin-angiotensin system*. Robert, J.I.S. & Nicholis, M.G.(Eds.), Gower Medical Publishing, London, UK. 1993.

8. DiBona GF. Nervous kidney: interaction between renal sympathetic nerves and renin-angiotensin system in the control of renal functions. Hypertension. 2000; 36:1083-1088.

9. Münter K, Ehmke H, Kirchengast M. Am J Physiol 1999; 276(3 Pt 2):H1022-H1027.

10. Bayorh MA, Ganafa AA, Eatman D, Walton M, Feuerstein GZ. Simvastatin and losartan enhance nitric oxide and reduce oxidative stress in salt-induced hypertension. Am J Hypertens 2005; 18(11): 1496-1502.

11. Bayorh MA, Layas MF, Mann G, Feuerstein GZ, Eatman D. The effect of diet on simvastatin and losartan enhancement of endothelial function. Clin Exp Hypertens 2007; 29(5):311-325.

12. Armenia A, Munavvar AS, Abdulla NA, Helmi A, Johns E J. The contribution of adrenoceptor subtype(s) in the renal vasculature of diabetic spontaneously hypertensive rats. Bri J Pharmacol. 2004; 142:719-726.

13. Hye Khan MA, Sattar MA, Abdullah NA, Johns EJ. Cisplatin cause altered renal hemodynamics in rats: role of augmented a-adrenergic responsiveness. Exp Toxicol Pathol 2007; 53: 253-260.

14. Khan AH, Sattar MA, Abdullah NA, Johns EJ. Influence of cisplatin-induced renal failure on the alpha(1)-adrenoceptor subtype causing vasoconstriction in the kidney of the rat. Eur J Pharmacol 2007; 569: 110-118.

15. Bello-Reuss E, Colindres RE, Pastoriza-Munoz E, Mueller RA, Gottschalk CW. Effect of acute unilateral renal denervation in the rat. J Clin Invest 1975; 50: 206-217.

16. Wu XC, Johns EJ. Nitric oxide modulation of neurallyinduced proximal tubular fluid reabsorption in the rat. Hypertens 2002; 39: 790-798.

17. Le Fevre ME, Guild S-J, Ramchandra R, Barrett CJ, Malpas SC. Role of angiotensin II in the control of renal function. Hypertens 2003; 41: 583-591.

18. Dendorfer A, Raasch W, Tempel K, Dominiak P. Comparison of the vascular and antiadrenergic activities of four angiotensin II type 1 antagonists in the pithed rat. J Hypertens 2002; 20: 1151-1156.

19. Jippe CB, Marie-Jeane M, Martin P, Pieter AV. Sympatho-inhibitory actions of irbesartan in pithed spontaneously hypertensive and Wistar-Kyoto rats. Fund. & Clinical Pharmacol 2003;17 (1): 83-91.