—Regular Article—

A Special Focus on the Role of α_{1D}-Adrenoreceptors in the Assessment of Renal Tubular Sodium Re-absorptive Responses in Spontaneously Hypertensive Rats Subjected to High Sodium Diet

Raisa N. KAZI,*[,a] Munavvar A. SATTAR,[a] Nor A. ABDULLAH,[b] Md A. Hye KHAN,[c] Hassaan A. RATHORE,[a] Mohammed H. ABDULLA,[a] Ibrahim M. SALMAN,[a] and Edward J. JOHNS[d]

[a]*School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia,* [b]*Department of Pharmacology, Faculty of Medicine, Universiti Malaya, 50603 Kuala Lumpur, Malaysia,* [c]*Department of Pharmacology and Toxicology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, Wisconsin 53226, USA, and* [d]*Department of Physiology, Aras Windle, University College Cork, College Road, Cork, Ireland*

(Received May 16, 2010; Accepted October 25, 2010)

α_{1D}-Adrenoceptors are involved in the genesis/maintenance of hypertension in spontaneously hypertensive rats (SHR). This study aims to investigate the role of α_{1D}-adrenoceptors in the antinatriuretic and antidiuretic responses in SHR subjected to high sodium (SHRHNa) and normal sodium (SHRNNa) intake for six weeks. Renal inulin clearance study was performed in which the antinatriuretic and antidiuretic responses to phenylephrine were examined in the presence and absence of α_{1D}-adrenoceptors blocker BMY7378. Data, mean±S.E.M. were subjected to ANOVA with significance at $p<0.05$. Results show that feeding SHR for six weeks with high salt did not cause any change in blood pressure. SHRHNa had higher (all $p<0.05$) urine flow rate (UFR), fractional and absolute excretion of sodium (FE_{Na} and $U_{Na}V$) compared to SHRNNa. Phenylephrine infusion produced significant reduction in UFR, FE_{Na} and $U_{Na}V$ in both SHRHNa and SHRNNa. The antidiuretic and antinatriuretic responses to phenylephrine in both groups were attenuated in the presence of BMY7378. Moreover, the antidiuretic and antinatriuretic responses to phenylephrine and BMY7378 were independent on any significant changes in renal and glomerular hemodynamics in both groups. Thus we conclude that high sodium intake did not bring any further increase in blood pressure of SHR, however, it results in exaggerated natriuresis and diuresis in SHRHNa. Irrespective of dietary sodium changes, α_1-adrenoceptors are involved in mediating the antinatriuretic and antidiuretic responses to phenylephrine in SHR. Further, high sodium intake did not significantly influence the functionality of α_{1D}-adrenoceptors in mediating the adrenergically induced antinatriuresis and antidiuresis.

Key words——α_{1D}-adrenoceptor; high sodium; renal function; spontaneously hypertensive rat (SHR)

INTRODUCTION

High dietary salt intake has long been associated with high blood pressure. Chronic exposure to a high-salt diet appears to be a major pathophysiological factor involved in the frequent occurrence of hypertension.[1,2] Hypertension is a condition where in adrenergic responsiveness, sympathetic nervous system activity and alpha adrenoceptors remains altered.[3,4] Renal α_1-adrenoceptor has shown to mediate the actions of renal sympathetic nerve in the control of the various renal functions such as renal hemodynamic, glomerular ultra filtration and sodium reabsorption, thus contributing to the regulation of extracellular fluid volume and arterial blood pressure. In addition, the release of renin from the granular cells of the juxtaglomerular apparatus is also mediated by renal α_1-adrenoceptors.[5]

Renal α_1-adrenoceptor is of primary pathogenic importance in hypertension[6] and dietary sodium intake has shown to exert a role in the regulation of renal α_1-adrenoceptors in the essential hypertension.[7–9] α_1-Adrenoceptors are subdivided into three distinct subtypes, α_{1A}, α_{1B} and α_{1D}.[10] Among the subtypes of α_1-adrenoceptors, α_{1D}-adrenoceptors is expressed in several vascular beds including the renal vasculature in spontaneously hypertensive rats (SHR) and are actively involved in the regulation of renal vascular resistance.[11] α_{1D}-Adrenoceptors is also suggested to be functionally important in the genesis and maintenance of essential hypertension thus contributing to the pathogenesis of hypertension.[11–13] The fact evi-

*e-mail: raisakolhar@yahoo.co.in

denced by a study done by Tanoue and coworkers, where in the genetic disruption of α_{1D}-adrenoceptor gene in mice resulted into hypotensive effects.[7] Reports also suggested that prehypertensive SHR have augmented basal amounts of α_{1D}-adrenoceptor mRNA and protein as compared to those amounts observed in normotensive Wistar Kyoto rats.[14] In addition, a major role of α_{1D}-adrenoceptor in the vascular smooth muscle cells in the development of salt induced hypertension in mice has been confirmed by gene knockout studies.[7] Moreover, the role of α_{1D}-adrenoceptor in the regulation of renal hemodynamic has been documented in several pathological conditions and in normotensive Wistar Kyoto rats subjected to high sodium diet.[8,11,15,16]

Reports regarding the possible role of α_{1D}-adrenoceptor in the peripheral vascular resistance in the genesis and maintenance of salt induced blood pressure responses have been established. While there is paucity regarding the role of α_{1D}-adrenoceptor in the regulation of tubular sodium reabsorption (a key determinant of blood pressure regulation and extracellular fluid volume) in essential hypertension when subjected to dietary sodium changes. So, this study is focusing on the role of α_{1D}-adrenoceptor in the regulation of renal tubular antinatriuresis and diuresis in essential hypertensive rat model subjected to high sodium diet for six weeks.

METHODS AND MATERIALS

Male SHR with body weights that were within the range of 250–300 g were maintained in the animal care facility, Universiti Sains Malaysia, Penang, Malaysia. In addition, animal handling and all procedures on animals were approved by animal ethics committee, Universiti Sains Malaysia, Penang, Malaysia. Prior to the assessment of renal functional studies, SHR with normal sodium intake (SHRNNa) were given normal standard rat chow (Gold Coin Feedmills Sdn Bhd, Malaysia) and tap water *ad libitum*. High-sodium-intake SHR (SHRHNa) were given normal standard rat chow and supplemented with 0.9% NaCl in drinking water *ad libitum*.[17] Once SHR had completed the high sodium and normal sodium diet feeding schedule of six weeks they were subjected to acute renal tubular functional study.

Surgical Preparation for Renal Functional Studies

Animals were starved overnight and anaesthetized with an intraperitoneal injection of 60 mg/kg sodium pentobarbitone (Nembutal®, CEVA Sante Animale, Liboure, France). The trachea was cannulated with endotracheal cannula (PP240, Portex, Kent, UK) to provide a clear airway passage. The left jugular vein was cannulated (PE 50, Portex, Kent, UK) to allow supplementary injections of 2 ml of inulin (10 mg/ml) in saline as a primer. The right carotid artery was cannulated for the measurement of systemic arterial blood pressure using a pressure transducer (P23 ID Gould, Statham Instrument, UK) coupled to a computerized data acquisition system (PowerLab®, ADInstruments, Sydney, Australia) for continuous measurement of mean arterial pressure.

The left kidney was exposed *via* a midline abdominal incision and the abdominal contents were carefully moved to the right side of the body. A cannula (PE 50, Portex, Kent, UK) was inserted into the left iliac artery and was advanced into the abdominal aorta, such that its tip lay at the level of the renal artery to enable the infusion of saline and also administration of all drugs to be given close renal arterially. The left ureter was cannulated (PE 10, Portex, Kent, UK) to enable collection of urine. Further in the experiments approximately 1 to 1.5 cm of the aorta was cleared and a screw-controlled snare was placed around it above the renal arteries. The snare was slightly tightened to lower blood pressure distal to the constriction site if systemic pressure rise as a consequence of adrenergic agonist infusion and might affect the kidney functions. Later during the experiments the snare could be released or tightened as needed, and the mean blood pressure below the snare, equivalent to renal arterial pressure was maintained constant. Once the completion of the surgical process, 2 ml of inulin (10 mg/ml) in saline (150 mM NaCl) was given as a primer *via* the jugular vein cannula and an infusion of saline containing inulin 10 mg/ml and sodium pentobarbitone (12.5 mg/kg/h) was begun at a rate of 6 ml/h *via* the iliac arterial cannula.[18]

Experimental Protocol for Renal Tubular Study

Once the completion of the surgery, the animals were allowed to stabilize for 1 h. After the stabilization period, a three-phase experiment were started and each phase lasted for 2 h. Phase 1 was the control (saline) phase, while phase 2 was phenylephrine-administration phase during which a dose of (100 μg/kg/h) of phenylephrine was infused intrarenally *via* the iliac artery cannula. Likewise, phase 3 involved the administration of the same dose of phenylephrine;

however, it was carried out in the presence of BMY7378 at a dose of (200 μg/kg/h). Baseline levels of mean arterial blood pressure, renal cortical perfusion and renal arterial pressure were recorded at the beginning of each phase. Arterial blood samples (400 μl) were withdrawn at the beginning and at the end of each pair of clearances from the carotid cannula into a precooled syringe, centrifuged for 2 min (6000 rpm) and the plasma removed. The remaining packed blood cells were resuspended in an equal volume of saline and reinfused into the animal within 5 min. The clearance period was started 5–10 min after the reinfusion of the blood sample when the cardiovascular variables had settled.[18,19] During each phase, three 15-min urine clearances samples were collected and the volumes obtained during each clearance period were measured gravimetrically and UFR is subsequently calculated. Further, plasma and urine samples were assayed for inulin using the modified method[20] and the glomerular filtration was calculated as the clearance of inulin.[21] Plasma and urine electrolytes were measured by flame photometry (Jenway Ltd, Fetsted, Essex, UK) followed by the $U_{Na}V$ and FE_{Na} calculation.

Measurement of Mean Arterial Blood Pressure, Renal Cortical Perfusion and Renal Arterial Pressure during Renal Tubular Functional Studies Local renal cortical perfusion was measured by needle (implantable) Laser Doppler flow meter. The probe is connected to a power lab system (Powerlab®, ADInstruments, Sydney, Australia). The mean arterial pressure was recorded through the carotid artery that was cannulated and connected to a fluid filed pressure transducer coupled to a computerized data acquisition system (PowerLab®, ADInstruments, Sydney, Australia). The iliac artery was also cannulated and connected to a fluid filed pressure transducer coupled to a computerized data acquisition system for continuous measurement of renal arterial pressure. The renal arterial pressure was maintained at a constant level throughout to minimize the potential effects of pressure on kidney function.

Drugs Used α_{1D}-Adrenoceptor antagonist BMY-7378-(2-[4-(2-methoxyphenyl)-1-iperazinyl]ethyl)-8-azaspiro[4.5]decane-7,9-dione (Research Biochemical Inc., UK) used in the present study is a selective α_{1D}-adrenoceptors antagonist and phenylephrine (Boots Co. LTd., UK) being the non specific α_1-adrenoceptor agonist.[15] These drugs were prepared in normal saline and kept frozen as stocks. Fresh working dilutions were made from the stock solution in normal saline before the start of each experiment. The dose of BMY7378 and phenylephrine used in the present study were 200 μg/kg/h and 100 μg/kg/h. The rationale of the use of proposed dose of the drug to carry out renal tubular function in the study is based previous studies from our laboratory and others.[8,15,18]

Statistical Analysis The renal responses in all the three phases were measured by taking the average value of the three clearances in each phase. All data were expressed as means±S.E.M. The renal functional responses for control, agonist and antagonist were compared between the phases (saline, agonist and antagonist treated phases). Statistical analysis was performed by one-way ANOVA on repeated measures (Superanova, Abacus Inc., CA, USA) followed by Bonferonni post-hoc test. Differences between the means were considered significant at the 5% level.

RESULTS

High sodium intake in SHR did not bring any significant change to baseline level of mean arterial pressure as compared to normal sodium diet (Table 1). Urine flow rate (UFR), fractional sodium excretion (FE_{Na}) and absolute sodium excretion ($U_{Na}V$) during the control (saline) phase in SHRHNa were higher (all $p<0.05$) compared to the same phase in SHRNNa. Phenylephrine infusion in the second phase produced reduction (all $p<0.05$) in the UFR, FE_{Na} and $U_{Na}V$ when compared to their corresponding control phase in both SHRNNa and SHRHNa groups (Fig. 1). In addition, we observed that phenylephrine infusion in the second phase is associated with significant ($p<0.05$) decrease in the renal cortical perfusion compared to their corresponding control phase in SHRHNa group, similarly there was a reduction in the glomerular filtration and renal cortical perfusion compared to their corresponding control phase in the SHRNNa group, but this decrease is not statistically significant. Results also show that phenylephrine infusion did not bring any significant change in the mean arterial pressure and renal arterial pressure in the second phase when compared to saline phase (Table 1).

In the third phase, BMY7378 is infused in presence of phenylephrine. BMY7378 infusion did attenuate

Table 1. Systemic and Renal hemodynamic Parameters measured during renal functional studies in SHR subjected to dietary sodium changes

Group	SHRNNa			SHRHNa		
	Phase			Phase		
Parameter	1	2	3	1	2	3
Mean arterial pressure (mmHg)	136.0±2.4	133.5±2.5	125.7±3.7*	132.2±1.8	132.6±4.5	125.6±6.7
Renal arterial pressure (mmHg)	132.0±4.8	136.0±2.6	129.5±6.3	135.2±1.9	133.2±4.9	125.0±7.7
Renal cortical perfusion (BPU/min)	190.7±23.1	144.0±18.5	190.5±20.8	200.2±19.5	156.6±5.6*	189.6±6.9
Glomerular filtration rate (ml/kg/min)	2.4±0.6	1.7±0.9	2.1±0.7	2.4±2.3	1.9±0.4	2.3±1.1

Glomerular filtration rate, mean arterial pressure, renal arterial pressure and renal cortical perfusion in SHRNNa and SHRHNa rats in phase 1 control (saline), phase 2 agonist (phenylephrine, 100 μg/kg/h) and phase 3 antagonist (BMY7378, 200 μg/kg/h) in presence of agonist (phenylephrine, 100 μg/kg/h). Values are expressed as mean±S.E.M., *$p<0.05$ compared to saline phase.

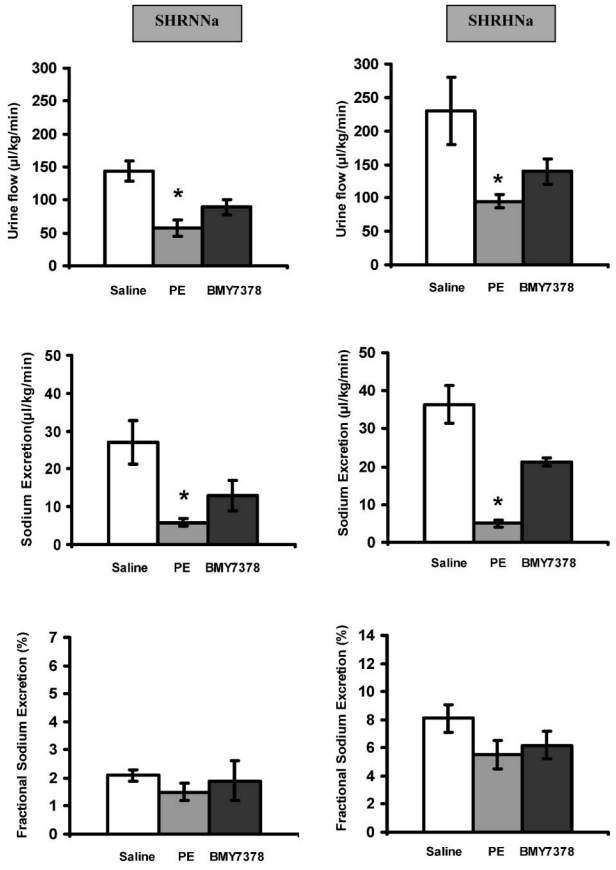

Fig. 1. Renal Tubular Functional Parameters measured during renal functional studies in SHR subjected to dietary sodium changes

Urine flow rate (UFR), absolute sodium excretion ($U_{Na}V$) and fractional excretion of sodium (FE_{Na}) during vehicle, phenylephrine infusion and BMY7378 infusion in presence of phenylephrine in SHRNNa and SHRHNa group. Data presented as mean±S.E.M. *$p<0.05$ compared to saline phase.

the antinatriuretic and antidiuretic response in the third phase in both SHRNNa and SHRHNa groups, but this inhibition was not statistically significant (Fig. 1). BMY7378 infusion in the third phase did not bring any significant change in the renal and glomerular hemodynamics when compared to phase one or phase two. Further, we observed that there was a significant decrease in the mean arterial pressure in the third phase compared to corresponding saline phase in SHRNNa group (Table 1).

DISCUSSION

High dietary sodium intake is associated with abnormal increase in renal sympathetic nerve activity, leading to an increase in peripheral vascular resistance in essential hypertension.[22,23] The aim of the present study is to provide information on the role of α_{1D}-adrenergic receptor in the regulation of antidiuretic and antinatriuretic responses in essential hypertensive rat subjected to high sodium load.

Our data demonstrate that SHR on high sodium diet show exaggerated increase in the UFR, $U_{Na}V$ and FE_{Na} compared to their control counter parts. Similar results observed by other researchers have suggested that the accentuated reflex inhibition of renal sympathetic activity *via* cardiopulmonary baroreceptor reflex activation and decreased tubular sodium reabsorption upon volume loading explains the exaggerated natriuresis and diuresis in essential hypertension.[24,25] In addition, reports also suggest that a significant increase in renal interstitial hydrostatic pressure after saline loading is responsible for the associated increases in the natriuretic and diuretic response.[26] Further, in the present study, phenylephrine, a non selective α_1-adrenergic agonist which stimulates all the three α_{1A}-, α_{1B}- and α_{1D}-adrenoceptor subtypes when infused leads to a significant reduction in $U_{Na}V$ and FE_{Na} in both SHRHNa and SHRNNa groups. In the present study, renal arterial pres-

sure remained constant so that major effect on renal tubular functional responses is avoided. In addition, phenylephrine infusion did not cause any significant change in mean arterial pressure or renal arterial pressure. Although phenylephrine infusion in this study was associated with a decrease in glomerular filtration and renal cortical perfusion in both SHRHNa and SHRNNa groups, but this reduction did not reach any statistically significant level and thus may have not been responsible for the observed antinatriuresis and antidiuresis. Further, studies have shown that up to 15% to 20% decrease in the glomerular hemodynamic had only a little influence on the magnitude of the associated antinatriuresis and antidiuresis.[18,19,27] Similarly, we observed a more significant decrease in the renal cortical perfusion in SHRHNa group in response to phenylephrine infusion. Moreover, renal cortical perfusion does not represent the total blood flow to the kidney as we have not measured the medullary blood flow. Thus the antinatriuretic and antidiuretic response to phenylephrine observed in the present study could be due to a direct action of the agonist on the tubular epithelial cells and not due to change in the glomerular hemodynamic. Thus these observations suggest that irrespective of dietary sodium changes, α_1-adrenoceptors are involved in mediating the renal tubular antinatriuretic and antidiuretic response in SHR. This study further strengthens the earlier observations which suggest that α_1-adrenoceptors play a major role in mediating the renal tubular antinatriuretic and antinatriuretic response to renal nerve stimulation as well as to the infusion of α_1-adrenoceptor agonist.[18,19,27,28] Further, BMY7378 infused close renal arterially only slightly attenuated the antinatriuretic and antinatriuretic response of phenylephrine but the values did not reach the control levels. BMY7378, a specific α_{1D}-adrenoceptor antagonist fails to significantly abolish the antinatriuresis and antidiuresis observed in response to phenylephrine infusion in the second phase of the experiment. These reasoning led us to understand that α_{1D}-adrenergic receptor has no significant role in the antinatriuretic and antidiuretic response in SHRHNa. It was also observed that in SHRNNa group, BMY7378 infusion leads to a significant decrease in the mean arterial pressure, the reason of which remains unclear. However this decrease in the mean arterial pressure did not bring any major effect on the renal arterial pressure, renal cortical perfusion and glomerular filtration and thus may have not affected the kidney function. Thus our finding's hypothesis that high sodium diet in SHR did not influence on the functionality of α_{1D}-adrenergic receptor in the regulation of renal tubular sodium reabsorptive responses.

CONCLUSION

Thus it is concluded that SHR on high sodium diet showed exaggerated increase in the diuresis and natriuresis. Irrespective of dietary sodium changes α_1-adrenoceptors are responsible for the antidiuretic and antinatriuretic response to phenylephrine in SHR. In addition, the α_{1D}-adrenergic receptor does not play any significant role in the regulation of renal tubular sodium reabsorptive responses in SHR subjected to high sodium diet.

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