Antidiuretic and antinatriuretic response to high salt load in normotensive Wistar-Kyoto rats: Role of alpha-1A-adrenoceptors

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Summary
1. Altered renal adrenergic responses have been recognized as pathophysiological responses to high salt intake. This study aims to investigate the influence of 6 weeks of high salt diet on α1A-adrenoceptor regulation of renal tubular antinatriuretic and antidiuretic response in normal Wistar Kyoto rats.
2. To achieve the above objective, antinatriuretic and antidiuretic response to phenylephrine was measured in the absence and presence of 5-methylurapidil (5-MeU) using the inulin clearance method. Systemic mean arterial blood pressure and renal haemodynamics were also measured simultaneously.
3. Six weeks of high salt intake in Wistar-Kyoto (WKY) rats did not bring any significant increase in mean arterial blood pressure. WKY rat on high salt diet (WKYHNa) showed an exaggerated increase in absolute and fractional sodium excretion. There was a significant involvement of α1A-adrenoceptor in carrying out renal tubular antinatriuretic and antidiuretic response in Wistar Kyoto rats on normal sodium diet (WKYNa). However, α1A-adrenoceptor played a minimal role in handling the tubular reabsorptive response in WKY rats on high salt diet.

KEYWORDS
antidiuresis, antinatriuresis, high salt, WKY rats, α1A-adrenoceptor

1 | INTRODUCTION

Chronic high salt intake has shown to influence the activity of the renal adrenergic system resulting into abnormal renal function and cardiovascular diseases. Salt sensitivity in essential hypertension is linked to an enhanced sympathetic activity as evidenced by increase in renal sympathetic nerve activity and increased circulating concentrations of catecholamines.1,2

The renal sympathetic nerve is considered to be important in regulating renal haemodynamic and tubular function. These functional effects contribute significantly to the renal regulation of arterial blood pressure and cardiovascular homeostasis.2 The renal sympathetic nerve densely innervates all the components of the kidney mediating its action through renal α- and β-adrenoceptor.3 Regarding the subtypes of α-adrenoceptor, α1-adrenoceptors mediate renal sympathetic regulation of renal vascular resistance and tubular electrolyte reabsorption.4 α1-adrenoceptors are subdivided into three distinct subtype, α1A, α1B and α1D.5,6

Among the subtypes, the α1A-adrenoceptor is functionally relevant in the regulation of renal haemodynamics.4,7 Studies have demonstrated that an increase in the renal sympathetic nerve activity decreases the renal blood flow by activation of α1A-adrenoceptor.8 Furthermore, a dominant role of α1A-adrenoceptor has been suggested in the regulation of vasomotor tone.9 Extensive research has led to a proposed role of α1A-adrenoceptor in the regulation of renal haemodynamic and tubular function under different pathological and dietary conditions. In relation to this, reports have suggested that mean arterial pressure (MAP) induced by phenylephrine was mainly...
mediated by $\alpha_{1A}$-adrenoceptor in both the anesthetized Wistar rats and spontaneous hypertensive rats (SHR). Evidence also shows the involvement of $\alpha_{1A}$-adrenoceptor in mediating the adrenergic regulation of tubular Na+ reabsorption in the spontaneously hypertensive stroke prone rat (SHRSP). During the phase of increasing blood pressure in SHR, $\alpha_{1A}$-adrenoceptor increases sodium transport in the distal tubule. Chronic stimulation of renal nerves or intrarenal infusion of noradrenaline increases sodium retention leading to hypertension which can be attenuated by renal denervation. The role of $\alpha_{1A}$-adrenoceptor subtype in the control of renal vasculature was enhanced in Sprague Dawley rats exposed to fructose feeding. Regarding the influence of high salt intake on the renal adrenergic system, studies have reported significant elevation in renal $\alpha_1$-adrenoceptor density after a high sodium diet in normotensive and hypertensive conditions as evidenced by its effect on renal haemodynamics and tubular sodium handling. Furthermore, it has been observed that high sodium diet has increased the functional contribution of $\alpha_{1A}$-adrenoceptor both in renal cortical vascular resistance, tubular Na+ and water reabsorption in spontaneously hypertensive rats. Previously, our studies have demonstrated that $\alpha_{1A}$-adrenoceptor is the major subtype involved in renal cortical vasculature and renal haemodynamics in normotensive Wistar-Kyoto rats (WKY) fed on a high sodium diet. However, the potential effect of high salt intake on renal tubular absorptive response of $\alpha_{1A}$-adrenoceptor under normotensive conditions remained unexplored. Against this background, this study hypothesized that hypertensinogenic stimulus such as high salt intake could bring alteration in the functional contribution of $\alpha_{1A}$-adrenoceptor in handling the antidiuretic and antinatriuretic response in normotensive WKY. This study was conducted to assess renal tubular absorptive response of $\alpha_{1A}$-adrenoceptor in normotensive WKY when subjected to high salt diet for 6 weeks and compared it to rats on normal sodium diet.

2 | METHODS

2.1 | Animals

Male WKY, 250-300 g, were maintained in the Animal Care facility of University Sains Malaysia, Penang, Malaysia. Animal handling and all other procedures were approved by the Animal Ethics Committee, University Sains Malaysia, Penang, Malaysia. WKY rats were maintained for 6 weeks on normal sodium diet of standard rat chow and tap water to drink (WKYNa, n=12) or a high sodium intake (W KYHNa, n=12) where rats were given isotonic saline (NaCl, 9 g/L) to drink ad libitum.

2.2 | Surgical preparation of animals

Rats were fasted overnight (with water ad libitum) before being anesthetized with 60 mg/kg (ip) sodium pentobarbitone (Nembutal; CEVA Sante Animale, Libourne, France). After tracheotomy with an endotracheal cannula (PP240; Portex, Kent, UK), the carotid artery was cannulated (PE 50; Portex) and connected to a pressure transducer (P23 ID Gould; Statham Instrument, Nottingham, UK) coupled to a computerized data acquisition system (PowerLab®; ADInstruments, Sydney, Australia) for continuous measurement of MAP. The left jugular vein was cannulated (PE 50; Portex) to permit infusion of maintenance doses of anaesthetic. Following a midline abdominal incision, the left kidney was exposed. Then, a cannula (PE 50; Portex) was inserted via the iliac artery to the level of the renal artery of the left kidney to enable exogenous administration of adrenergic agonists and antagonist through the renal artery into the kidney. The cannula was kept patent by continuous infusion of saline at a rate of 6 mL/h. The left ureter was cannulated for collection of urine. Renal cortical perfusion was measured by Needle (implantable) Laser Doppler flow meter. The probe was connected to a PowerLab® (AD instruments); 2 mL of Inulin (10 mg/mL) in saline was given as a primer via the jugular vein cannula followed by the continuous infusion of saline containing Inulin (10 mg/mL) and sodium pentobarbitone (12.5 mg/kg/h) at a rate of 6 mL/h. The iliac artery was cannulated, and the cannula was connected to a pressure transducer (P23 ID Gould; Statham Instrument) coupled to a computerized data acquisition system (PowerLab®; ADInstruments) for continuous measurement of renal arterial pressure (RAP). A screw-controlled snare was placed around the aorta above the renal arteries, and blood pressure was regulated to avoid any increments in RAP in response to the vasoactive agent (Figures 1 and 2).

2.3 | Experimental protocol

Following completion of the surgery, the animals were allowed to stabilize for 1 hour. The experiment was divided into three phases with the first part being the control (vehicle control, saline) phase. In the second phase, PE was infused at a dose of 100 μg/kg/h close renal arterially. In the third phase, PE infusion was carried out (100 μg/kg/h) in the presence of a continuous infusion of 5-methylurapidil (5-MeU) at a dose of 10 μg/kg/h close renal arterially. Each of these experimental phases consisted of a series of three 15-minutes urine clearance periods. Blood samples were taken at the beginning and at the end of each clearance period. Arterial blood samples (~400 μL) were withdrawn from the carotid cannula into a precooled syringe, centrifuged for 2 minutes (400 g), and plasma was separated and kept frozen (~800°C) until analysed. The remaining blood cells were re-suspended in an equal volume of saline and re-infused into the animal within 5 minutes. The clearance period was started 5-10 minutes after the re-infusion of the blood sample, when the cardiovascular variables had settled. The urine produced during each clearance period was measured gravimetrically. Plasma and urine samples were assayed for inulin. Glomerular filtration rate was calculated as the clearance of inulin. Plasma and urine electrolytes were measured by flame photometry (Table 1).

2.4 | Drugs used

The relatively selective antagonist of $\alpha_{1A}$-adrenoceptor, 5-MeU (Research Biochemical International, Natick, MA, USA), utilized in this
The study was chosen based on the pharmacological selective affinity of the drug at α₁-adrenoceptor subtypes. Phenylephrine (BASF Pharm, Knoll, UK) is a non-selective synthetic agonist of α₁-adrenoceptors with an ability to activate all three α₁A-, α₁B-, and α₁D-adrenoceptor subtypes. Reports show that phenylephrine has higher affinity for the α₁A- and α₁D-adrenoceptors than α₁B-adrenoceptor.

2.5 | Statistics

The renal excretory responses in all the three phases were measured by taking the average value of three clearances in each phase. All data were expressed as the mean ± SEM. The renal functional response for control, agonist and antagonist was compared between the phases (saline-, agonist- and antagonist-treated phases). Statistical analysis was performed by one-way ANOVA on repeated measures (Super ANOVA; Abacus Concepts, Inc., Berkely, CA, USA) followed by the Bonferroni post hoc test. Differences between the means were considered significant at the 5% level. The absolute and percentage changes quoted in the text represent the mean value calculated from individual rats.

3 | RESULTS

Wistar-Kyoto rats on high salt diet for 6 weeks did not show any significant increase in mean arterial blood pressure. The study was conducted in three phases. During the first phase of acute renal
functional study, urine flow rate (UFR), absolute sodium excretion (UNaV) and fractional sodium excretion (FENa) were measured under saline infusion in WKYNNa and WKYHNa diet rats. Following this, in the second phase, the tubular reabsorptive responses were measured when phenylephrine was infused close renal arterially into the kidney. Additionally, glomerular filtration rate (GFR), mean arterial blood pressure, renal arterial blood pressure and renal cortical perfusion were also measured in all the three phases. No significant changes were observed in the haemodynamic parameters between the three phases in both the experimental group. It was observed that there was a significant reduction in the UFR, UNaV and FENa in the second phase in response to phenylephrine in both WKYNNa and WKYHNa diet. In the third phase of the experiment, phenylephrine was infused in the presence of specific antagonist 5-MeU. 5-MeU significantly abolished antinatriuretic and antidiuretic effect of phenylephrine in WKYNNa diet. On the other hand, 5-MeU failed to attenuate the antinatriuretic and antidiuretic effect in WKYHNa diet rats significantly.

4 | DISCUSSION

Phenylephrine being a non-selective $\alpha_1$-adrenoceptor agonist known to stimulate all the three subtypes of $\alpha_1$-adrenoceptor. In the present study, phenylephrine was infused into the kidney in the absence and presence of selective $\alpha_{1A}$-adrenoceptor antagonists 5-MeU, to categorize $\alpha_1$-adrenoceptor subtypes involved in mediating the tubular function. The route of drug administration chosen was close to the renal artery in an attempt to maximize the local effect on the kidney and to minimize systemic spillover. WKY on high sodium diet showed an exaggerated increase in the sodium excretion and fractional sodium excretion in the control saline phase, when compared to WKY on normal sodium diet. The phenomenon of diuresis and natriuresis after salt loading can be described as a complex renal autoregulatory mechanism involving the neural and hormonal mechanisms. It is considered to be one of the most basic and powerful mechanism to achieve haemostasis. During changes in sodium and fluid intake, this feedback mechanism helps to maintain fluid balance and minimize changes in blood volume, extracellular fluid volume and arterial pressure. The phenomenon of diuresis and natriuresis was independent of any increase in the systemic arterial blood pressure in WKY rats loaded with high salt. Similar responses reported by other studies suggest exaggerated diuresis and natriuresis during sodium loading is independent of any changes in the glomerular and renal haemodynamic. In relation to this, recent reports have suggested that renal nitric oxide synthesis plays a role in acute and chronic regulation of sodium balance. The endogenous nitric oxide participates in the renal adaptation to increased dietary salt intake, facilitating sodium excretion and allowing maintenance of normal blood pressure, but the exact mechanism is poorly understood, and moreover, the present study was not designed to determine these detailed mechanisms. In the present study, phenylephrine infusion to the renal artery led to a significant reduction in the urine volume, sodium excretion and fractional sodium, compared to the corresponding saline phase in both the experimental group. The renal artery infusion of phenylephrine in the second phase did not bring any major change in the MAP and RAP, but was sufficient to cause an antinatriuresis and antidiuresis, further strengthening the fact that, $\alpha_1$-adrenoceptor either by renal nerve stimulation or exogenously administered $\alpha_1$-adrenoceptor agonist, leads to antidiuresis and antinatriuresis.

In the third phase of the experiment, phenylephrine infusion was undertaken under the presence of specific $\alpha_{1A}$-adrenoceptors antagonist 5-MeU. 5-MeU more significantly attenuated the antinatriuretic and antidiuretic response of phenylephrine in WKYNNa diet rats as compared to WKY rats on high sodium diet, thus indicating that $\alpha_{1A}$-adrenoceptors are largely responsible for observed antinatriuresis and antidiuresis. These observations are in agreement with several earlier reports, which suggest the role of $\alpha_{1A}$-adrenoceptor in mediating antinatriuresis and antidiuresis. Infusion of phenylephrine and $\alpha_{1A}$-adrenoceptor antagonist 5-MeU did not bring about any significant change in the renal cortical perfusion, glomerular filtration rate,
mean arterial blood pressure and RAP. This response indicates that the antidiuretic and antinatriuretic response to phenylephrine is caused predominantly by α1A-adrenoceptors present on the tubular epithelial cells, but not by alterations in the systemic and renal haemodynamic.

This particular finding specifies that the α1A-adrenoceptors plays a major role in the observed antidiuresis and antinatriuresis in WKYNNa when compared to WKYHNa.

In conclusion, WKY rats on high sodium diet showed an exaggerated increase in the urine flow and sodium excretion. Irrespective of the changes in the dietary sodium intake, phenylephrine infusion is associated with marked reduction in urine flow, sodium excretion and fractional sodium excretion in WKY. Strengthening the earlier reports that antidiuretic and antinatriuretic response to exogenously administered α1-adrenoceptor agonist is caused predominantly by renal tubular α1-adrenoceptors. This study demonstrates that α1A-adrenoceptors are involved in the antidiuretic and antinatriuretic response at the renal tubular levels in WKYNNa diet rats. However, α1B-adrenoceptors have a minimal role in carrying out antidiuresis and antinatriuresis in WKYNNa diet rats. With regard to the hypothesis of the present study, high salt load in normotensive conditions has led to an altered α1a-adrenoceptor functional response in the regulation of renal tubular absorption. It is possible that functional involvement of other receptor subtypes in the renal adrenergic system is involved in the regulation of renal tubular function. This needs to be ruled out under normotensive condition, especially regarding the potential effects of high sodium diet.

CONFLICT OF INTEREST AND DISCLOSURE

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