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Influence of High Dietary Sodium Intake on Functional Contribution of Renal α_{1A} -Adrenoceptor of SHR^{*}

Wpływ dużej podaży sodu w diecie na czynnościowy wkład α_{1A} -adrenoceptora u szczurów z samoistnym nadciśnieniem tętniczym

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Abstract

Background. Elevated dietary sodium intake exacerbates the elevation of blood pressure in spontaneously hypertensive rats (SHR) which is associated with increased renal vascular resistance and fluid reabsorption. The renal sympathetic nerves exert their action via α_1 -adrenoceptors (α_1 -AR) with α_{1A} as the major subtype in regulating renal hemodynamics and excretory function.

Objectives. This study investigated whether the contribution of α_{1A} -AR in regulating renal vasoconstriction and excretory function was enhanced in SHR receiving high sodium intake.

Material and Methods. SHR received either water (SHRNNa) or normal saline (SHRHNa) for six weeks. Metabolic cage studies followed by acute experiments were carried out on these SHR to examine renal vasoconstrictor and excretory responses to catecholamine viz. noradrenaline (NA), phenylephrine (PE) and methoxamine (ME) in the presence and absence of a α_{1A} -AR blocker, 5-methylurapidil (5-MeU).

Results. Daily water intake and urine output were increased in SHRHNa ($P < 0.05$ vs. SHRNNa). Renal cortical vascular responses to NA, PE and ME were enhanced in SHRHNa ($P < 0.05$ vs. SHRNNa) and the greatest effect was seen with ME. Intra-renal infusion of PE caused antidiuresis and antinatriuresis. Irrespective of the sodium intake, 5-MeU markedly blunted adrenergically induced renal cortical vasoconstrictions in SHR, while the antinatriuretic and antidiuretic responses to PE were inhibited by 5-MeU in SHRHNa only ($P < 0.05$).

Conclusions. The data suggest that, irrespective of the level of dietary sodium intake, α_{1A} -ARs are the functional subtypes in SHR. However, there also appears to be a role for α_{1A} -AR in mediating enhanced renal cortical vascular resistance, augmented tubular Na^+ and water reabsorption in SHRHNa rats (Adv Clin Exp Med 2011, 20, 1, 47–55).

Key words: high salt, α_{1A} -adrenoceptors, kidney, SHR.

Streszczenie

Wprowadzenie. Zwiększone spożycie sodu nasila wzrost ciśnienia krwi u szczurów z samoistnym nadciśnieniem tętniczym (SHR), które jest związane z nasileniem oporu naczyniowego nerek i wchłanianiem zwrotnym płynu. Nerwy współczulne nerek wywierają swoje działanie przez α_1 -adrenoreceptory (α_1 -AR), a α_{1A} jest głównym podtypem w regulacji hemodynamiki nerek i czynności wydalniczych.

Cel pracy. Zbadanie, czy wkład α_{1A} -AR do regulacji zwężenia naczyń nerek i czynności wydalniczych został zwiększony u szczurów z samoistnym nadciśnieniem tętniczym spożywających dużo sodu.

Materiał i metody. Szczury otrzymywały albo wodę (SHRNNa), albo sól fizjologiczną (SHRHNa) przez sześć tygodni. Badanie w klatkach metabolicznych przeprowadzono po doświadczeniu ostrym w celu zbadania odpowiedzi zwężającej naczynia i wydalniczej nerek na katecholaminy, a mianowicie noradrenalinę (NA), fenylefrynę (PE) i metoksaminę (ME) w obecności i braku leku blokującego α_{1A} -AR i 5-methylurapidilu (5-Meu).

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Wyniki. Dzielne spożycie wody i wydalenie moczu było większe w grupie SHRHN (p < 0,05 vs SHRNN). Odpowiedź korowa naczyń nerek na NA, PE i ME była większa w grupie SHRHN (p < 0,05 vs SHRNN), a największy rezultat zaobserwowano pod wpływem ME. Śródnierkowy wlew PE wywołał antydiurezę i antynatriurezę. Niezależnie od spożycia sodu, 5-Meu wyraźnie ograniczył zwężenie naczyń krwionośnych wywołane adrenergicznie u szczurów z samoistnym nadciśnieniem tętniczym, a antynatriuretyczne i antydiuretyczne odpowiedzi na PE były hamowane przez 5-Meu tylko w grupie SHRHN (P < 0,05).

Wnioski. Dane wskazują, że niezależnie od poziomu spożycia sodu, α_{1A} -AR są czynnościowym podtypem u szczurów z samoistnym nadciśnieniem tętniczym. Wydaje się też, że swoją rolę spełnia α_{1A} -AR w mediacji zwiększenia oporu naczyń nerkowych warstwy korowej, wzbogacania cewkowego Na⁺ i reabsorpcji wody u szczurów SHRHN (Adv Clin Exp Med 2011, 20, 1, 47–55).

Słowa kluczowe: duże stężenie soli, α_{1A} -adrenoreceptory, nerka, szczury z samoistnym nadciśnieniem tętniczym.

Elevated dietary sodium intake has been associated with the development of hypertension, and has been shown to exacerbate the level of blood pressure in spontaneous hypertensive rats (SHR) [1–2]. There are a number of ways in which this can occur and one of which may be a change in the contribution of renal α_1 -adrenoceptors (α_1 -AR). There have been reports of a significant elevation in renal α_1 -AR density after a high sodium diet [3] together with augmented smooth muscle contractility and sodium reabsorption leading to enhanced renal vasoconstriction and sodium retention [4].

A raised dietary sodium intake has been reported to increase vascular smooth muscle responsiveness to catecholamine in stroke prone SHR [5]. Apart from animal models, this same phenomenon has also been shown in human, that there is an enhanced peripheral forearm vascular resistance response to sodium loading in borderline hypertensive patients [6]. Furthermore, it has been reported that adrenoceptor density and sensitivity can be altered in response to raised dietary sodium intake in normotensive subjects. Similarly, in a recent study, we reported that in normotensive Wistar Kyoto rats (WKY) subjected to a high salt intake, there was a raised renal cortical vascular responsiveness to α_1 -adrenergic agonists with a significant involvement of α_{1B} - along with α_{1A} - and α_{1D} -ARs in mediating adrenergically induced renal cortical vasoconstriction compared to control groups [7]. Among the different subtypes of α_1 -ARs, α_{1A} -AR has been shown to play a major role in the regulation of various renal functions including renal blood flow (RBF), glomerular filtration rate (GFR) and Na⁺ reabsorption [8–9]. Within the kidney, the density and functionality of α_{1A} -adrenoceptors has been consistently found to predominate over α_{1B} and α_{1D} [10–11] in normal rats and in rat models of disease including hypertension induced by various methods, a combined state of diabetes and essential hypertension, stroke-prone hypertensive (SPSHR), two kidney one clip (2K1C) and DOCA-salt hypertensive rats [12, 13]. It is also generally accepted that α_{1A} -ARs play a major role in mediating the adrenergic regulation of tubular Na⁺ reabsorption

in normotensive WKY and SHRSP [14]. In addition, gene expression studies have shown that the levels of gene expression for α_{1A} -adrenoreceptors were always higher, in all tissue samples investigated in SHR and is also well correlated with elevated blood pressure and increased sympathetic drive in SHR compared with both normotensive strains [15]. However, there is a paucity of information on the influence of high salt intake on the functional population of renal α_1 -ARs mediating the neural regulation of the kidney in the SHR.

In the present study, it was hypothesized that placing SHR's on a high dietary sodium intake would enhance the impact of the renal α_1 -ARs in the regulation of renal vascular resistance and tubular reabsorptive function. This was done by exposing SHR to either a normal or high dietary sodium intake and then determining the renal vasoconstrictor responses to a range of agonists and to a relatively selective α_{1A} -AR antagonist, 5MeU. Renal vasoconstrictor responses were assessed in the cortical vasculature, as it receives ~90% of the total blood volume flow to the kidney. Further, in these animals, tubular reabsorptive functions were also assessed in the presence and absence of high salt intake.

Material and Methods

Animals

Male spontaneously hypertensive rats (SHR), 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. SHR were maintained for six weeks with either a normal sodium intake of standard rat chow and given tap water to drink (SHRNN, n = 12) or a high sodium intake (SHRHNa, n = 12) where rats were given isotonic saline (NaCl, 9g/L) to drink *ad libitum*.

The animals were caged in commercially available metabolic cages. After a three-day acclimatization period, metabolic data collection commenced with baseline followed by experimental data collec-

tion for a period of six consecutive weeks. Tail-vein blood samples (250 µl) from the tail-tip were collected for biochemical analysis along with the collection of physiological data viz. body weight (Bwt), 24 h water intake (WI) and 24 h urine output (UO) once a week. Once the metabolic study terminated after the six-week treatment period, the rats were used in the terminal protocol comprising the acute renal vasoconstrictor or renal clearance study.

Assessment of Renal Cortical Vasoconstrictor Responses in SHR with Normal and High Sodium Intake (n = 6 in each group)

A series of adrenergic agonists, noradrenaline (NA), phenylephrine (PE) and methoxamine (ME) and the antagonist, 5-methylurapidil (5-MeU) were administered close-renal arterially to characterize the functional contribution of α_{1A} -AR subtypes in mediating adrenergically induced renal vasoconstrictor responses in SHR with normal (SHRNNa) and high (SHRHNa) sodium intake.

Surgical Preparation of Animals

Overnight fasted (with water *ad libitum*) rats were anesthetized with 60 mg/kg (i.p.) sodium pentobarbitone (Nembutal®, CEVA Sante Animale, Libourne, France). After tracheotomy with an endotracheal cannula (PP240, Portex, Kent, England), the carotid artery was cannulated (PE 50, Portex, Kent, England) and connected to a pressure transducer (P23 ID Gould, Statham Instrument, UK) coupled to a computerized data acquisition system (PowerLab®, ADInstruments, Australia) for continuous measurement of mean arterial pressure (MAP). The left jugular vein was cannulated (PE 50, Portex, Kent, England) to permit infusion of maintenance doses of anesthetic. Following a midline abdominal incision, the left kidney was exposed. Then a cannula (PE 50, Portex, Kent, England) 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 into the renal artery and hence the kidney. The cannula was kept patent by continuous infusion of saline at a rate of 6 ml/h [13].

Time Control Experiments (n = 6 in Each Group)

A series of time control experiments were carried out to examine any time dependent changes in the three different phases used in the experimental protocol. In this series of experiments, MAP and

superficial renal cortical blood perfusion (RCP) were measured in three different phases with an interval period that matched exactly the lapsed time between the three different phases used in the protocol with drug administration.

Experimental Protocol

On completion of the surgical preparation, baseline MAP and RCP were recorded. RCP was measured using a Laser Doppler flowmetry by placing a Laser Doppler flow probe on the surface of the kidney (PowerLab®, AD Instrument) and expressed in blood perfusion units (BPU). At the end of the experiment, the animals were killed by giving an overdose of anesthetic (sodium pentobarbitone; Nembutal®, CEVA Sante Animale, Libourne, France).

The renal vasoconstrictor experiment was carried out in three phases. In the first phase, saline was infused continuously and intra-renal arterially during which graded bolus doses of NA (25, 50, 100 and 200 ng), PE (0.25, 0.5, 1 and 2 µg) and ME (1, 2, 3 and 4 µg) were administered in an ascending and then descending manner. During the second phase, close intrarenal administration of a low bolus dose of 5-MeU (5 µg/kg) was given followed by a continuous infusion of 5-MeU (1.25 µg/kg/h) and, 15 minutes later the second set of vasoconstrictor responses were carried out with NA, PE and ME at the same dosages used in the first phase. In the last and third phase, a high bolus dose of 5-MeU (10 µg/kg) was administered followed by a continuous infusion of 5-MeU (2.5 µg/kg/h) and, 15 minutes later the last set of vasoconstrictor agonists were delivered as described in the first phase [13].

Assessment of Renal Excretory Functions in SHR with Normal and High Sodium Intake (n = 6 in Each Group)

Surgical Preparation of Animals

The surgical procedure for the functional study was similar to that of the renal vasoconstriction study, except that the left ureter was cannulated for collection of urine. 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, UK) coupled to a computerized data acquisition system (PowerLab®, ADInstruments, Australia) 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 renal perfusion pressure (RAP) in response to the vasoactive agent.

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 $\mu\text{g/kg/h}$ close renal arterially. In the third phase, PE infusion was carried out (100 $\mu\text{g/kg/h}$) in the presence of a continuous infusion of 5-MeU at a dose of 10 $\mu\text{g/kg/h}$ close renal arterially. Each of these experimental phases consisted of a series of three 15-minute 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 pre-cooled syringe, centrifuged for 2 min (6000 rpm) and plasma was separated and kept frozen (-80°C) until analyzed. The remaining blood cells were re-suspended in an equal volume of saline and re-infused into the animal within 5 min. The clearance period was started 5–10 minutes after the re-infusion of the blood sample when the cardiovascular variables had settled [14]. The urine produced during each clearance period was measured gravimetrically. Plasma and urine samples were assayed for Inulin using the method described earlier [16] and glomerular filtration rate was calculated as the clearance of Inulin [17]. Plasma and urine electrolytes were measured by flame photometry.

Drugs Used

The relatively selective antagonist of α_{1A} -ARs, 5-methylurapidil (5-MeU) (Research Biochemical's International, USA), NA (Levophed, Sanofi Winthrop, UK), PE (BASF Pharm, Knoll, UK) and ME (Vasoxine, Calmic Medical Division, UK) were diluted in saline (150 mmol NaCl) from stocks daily prior to use.

Statistical Analysis

The renal cortical blood perfusion responses to NA, PE and ME were taken as the average percentage reductions from baseline values caused by each dose of adrenergic stimuli administered in ascending and descending order. All data were expressed as mean % change $\pm$ S.E.M. of renal vasoconstrictor responses produced by all the doses (adrenergic agonists) and were compared between the phases (saline, low and high dose of antagonist treated phases). The data from the renal vasocon-

striction experiments were analyzed by a two-way ANOVA, while the metabolic data were analyzed by using a one-way ANOVA followed by the Bonferroni post-hoc test (SuperANOVA, Abacus Inc., CA, USA). The differences between the means were considered significant at the 5% level.

The renal excretory 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 $\pm$ 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 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.

Results

General Observations

Blood pressure measurements are presented as mean arterial pressure (MAP) recorded for 15 minutes prior to the commencement of the acute renal vasoconstrictor and renal tubular experiment. It was evident that there was no difference in the baseline levels of MAP between the normal (SHRNNa) and high sodium intake (SHRHNa) groups of SHR. Moreover, there was no change in RCP in SHRNNa following the administration of the low or high doses of 5-MeU during the renal vasoconstrictor experiment (Table 1). However, in both experimental groups the high dose of 5-MeU significantly ($P < 0.05$) depressed MAP in the third phase (Table 1). The mean values of 24 h WI, 24 h UO, Bwt and urinary excretion of sodium (UNa) were measured once a week for 6 weeks and were found to be greater in the SHRHNa as compared to the SHRNNa group (all $P < 0.05$) (Table 2). Moreover, the MAP measured at the end of the six-week treatment in the acute study was similar between the experimental groups (Table 2).

Time Control Experiments in SHR

In the time control experiments, the possible time dependent changes in MAP and RCP were measured in the presence of repeated infusion of saline at an interval period matching exactly the lapsed time between phases. There was no differ-

Table 1. Basal values of mean arterial pressure (MAP) and renal cortical blood perfusion (RCP) in different experimental phases in SHR treated with normal and high sodium diet (n = 6 in each group) *, P < 0.05 vs vehicle treated control phase. #BPU = blood perfusion unit

Tabela 1. Podstawowe wartości średniego ciśnienia tętniczego (MAP) i RCP w różnych fazach doświadczenia u szczurów, którym podawano dietę z normalną i dużą zawartością sodu (n = 6 w każdej grupie)

Experimental phases (Fazy doświadczenia)	SHR with normal Na ⁺ intake (SHR z normalną podażą Na ⁺)		SHR with high Na ⁺ intake (SHR z dużą podażą Na ⁺)	
	MAP (mm Hg)	RCP (BPU/min)	MAP (mm Hg)	RCP (BPU/min)
Phase 1 (Vehicle control) (Faza 1)	135 ± 3	178 ± 8	143 ± 3	199 ± 7
Phase 2 (Low dose of 5-Meu) (Faza 2)	112 ± 6	145 ± 10	112 ± 5	207 ± 21
Phase 3 (High dose of 5-Meu) (Faza 3)	103 ± 3 *	178 ± 27	103 ± 4*	171 ± 16

Table 2. Physiological data of SHR after six-week treatment with normal and high sodium diet. (n = 6 in each group). *, P < 0.05 vs SHR with normal sodium diet. BWt. – body weight, MAP – mean arterial pressure, WI – water intake, U_{Na} – urinary Na⁺, P_{Na} – plasma Na⁺, UO – urine output

Tabela 2. Dane fizjologiczne szczurów po 6 tygodniach stosowania diety z normalną i dużą zawartością sodu (n – 6 w każdej grupie). * p < 0,05 vs szczury z prawidłową ilością sodu w diecie. BWt. – masa ciała, MAP – średnie ciśnienie tętnicze, WI – spożycie wody, U_{Na} – zawartość Na⁺ w moczu, P_{Na} – zawartość Na⁺ w osoczu, UO – diureza

Treatment groups (Grupy badane)	BWt (g)	MAP (mm Hg)	WI (ml/24h)	UNa (mmol/l)	PNa (mmol/l)	UO (ml/24h)
SHR with normal Na intake	230 ± 3	135 ± 3	34 ± 5	48 ± 6	142.4 ± 6.3	21 ± 2
SHR with high Na intake	241 ± 1*	143 ± 3	76 ± 5*	84 ± 4*	132.6 ± 2.9	59 ± 4*

Table 3. Overall mean % change in renal cortical blood perfusion to adrenergic agonists in SHR with normal (n = 6) and high (n = 6) sodium intake. *, P < 0.05 vs control phase. #BPU = blood perfusion unit

Tabela 3. Ogółem średnia zmiana w % niewydolności korowego przepływu krwi do adrenergicznych agonistów u szczurów z normalnym (n = 6) i dużym (n = 6) spożyciem sodu. * p < 0,05 vs fazy kontrolnej, #BPU – jednostka perfuzji krwi

Adrenergic agonists (Agoniści receptorów adrenergicznych)	Renal cortical vascular responses – % reduction in cortical #BPU from baseline (Korowa odpowiedź naczyń nerek – % zmniejszenia korowej #BPU od stanu wyjściowego)					
	SHR with normal Na intake (SHRNNa)			SHR with high Na intake (SHRHNa)		
	control	5-MeU (5 µg/kg/h)	5-MeU (10 µg/kg/h)	control	5-MeU (5 µg/kg/h)	5-MeU (10 µg/kg/h)
Noradrenaline (Noradrenalin)	57 ± 3	49 ± 3	49 ± 3*	59 ± 3	54 ± 3	35 ± 3*
Phenylephrine (Fenyleferyna)	62 ± 4	47 ± 3*	33 ± 3*	65 ± 3	55 ± 4*	38 ± 3*
Methoxamine (Metoksamina)	35 ± 4	19 ± 2*	14 ± 1*	45 ± 3	20 ± 2*	13 ± 1*

ence in MAP (144 ± 3, 138 ± 3 and 143 ± 3 mm Hg) or RCP (207 ± 8, 201 ± 6 and 197 ± 6 BPU) between phases 1, 2 and 3.

Renal Cortical Vascular Response in SHR with Normal and High Sodium Intake

In the control phase, NA caused dose dependent renal cortical vasoconstrictions in both SHRNNa and SHRHNa groups (Table 3, Fig. 1–3).

The magnitudes of NA induced vasoconstrictions were markedly attenuated by the high doses of 5-MeU in SHRHNa and SHRNNa groups (all P < 0.05). Moreover, it was observed that this attenuation of NA induced vasoconstriction by 5-MeU was greater (P < 0.05) in SHRHNa compared to that of SHRNNa (Table 3, Fig. 1). An almost similar pattern of responses was observed for PE in both SHRHNa and SHRNNa with the exception that attenuations of the PE induced renal vasoconstrictor responses were caused by both doses of 5-MeU and in both experimental groups (Table 3, Fig. 2).

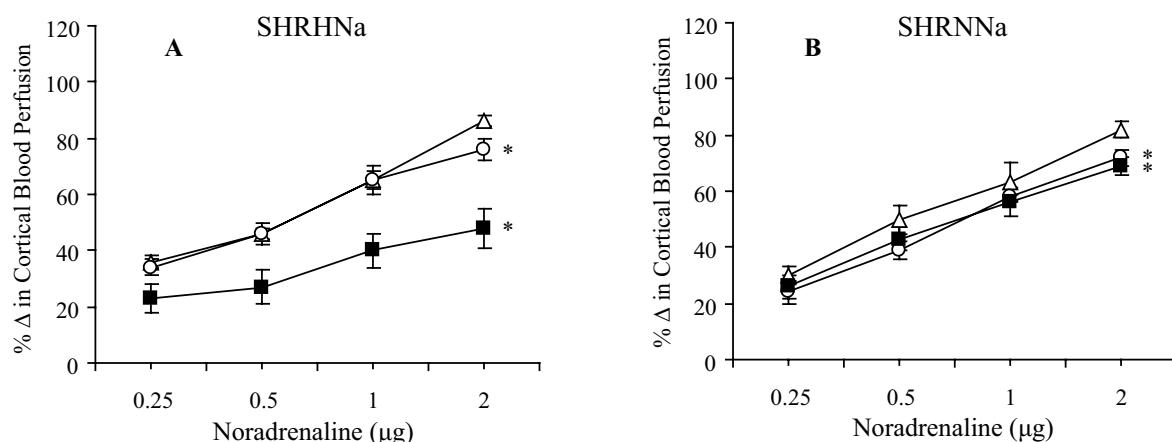


Fig. 1. Noradrenalin induced renal cortical vasoconstrictor responses in SHR with high (A) and normal (B) sodium intake in the presence and absence of 5-methylurapidil (Δ Saline, $\circ$ low dose and $\blacksquare$ high dose). * $P < 0.05$ vs. Saline, ($n = 6$).

Ryc. 1. Korowa odpowiedź zwężająca naczynia nerek wywołana noradrenaliną u szczurów z dużym ($n = 6$) i normalnym ($n = 6$) spożyciem sodu w obecności i braku 5-metylurapidilu (Δ sól fizjologiczna, $\circ$ mała dawka i $\blacksquare$ duża dawka). * $P < 0,05$ vs sól fizjologiczna ($n = 6$)

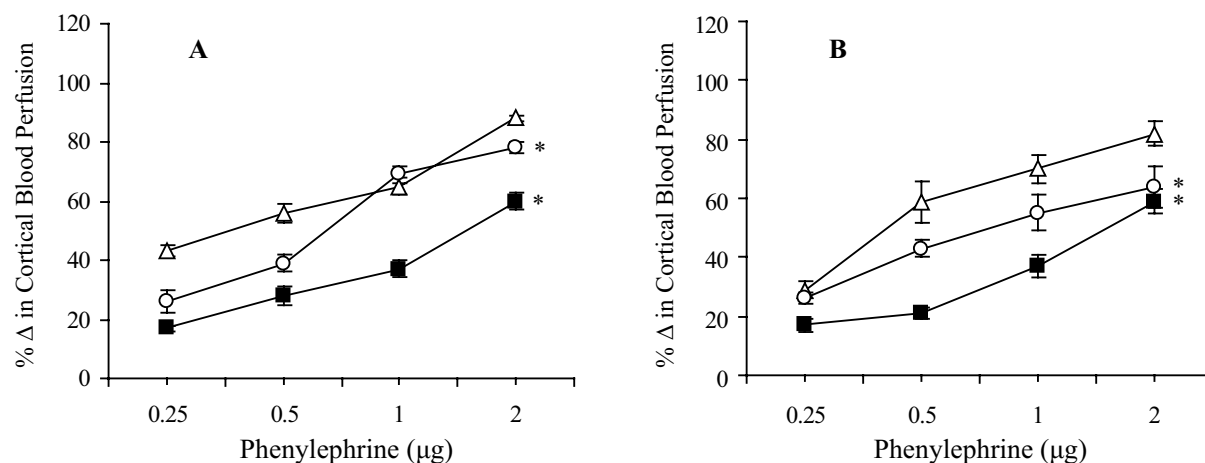


Fig. 2. Phenylephrine (PE) induced renal cortical vasoconstrictor responses in SHR with high (A) and normal (B) sodium intake in the presence and absence of 5-methylurapidil (Δ Saline, $\circ$ low dose and $\blacksquare$ high dose). *, $P < 0.05$ vs. Saline, ($n = 6$)

Ryc. 2. Korowa odpowiedź zwężająca naczynia nerek wywołana fenylefryną u szczurów z dużym ($n = 6$) i normalnym ($n = 6$) spożyciem sodu w obecności i braku 5-metylurapidilu (Δ sól fizjologiczna, $\circ$ mała dawka i $\blacksquare$ duża dawka). * $P < 0,05$ vs sól fizjologiczna ($n = 6$)

The magnitudes of the ME induced renal cortical vasoconstrictions in the control phase (in the absence of an antagonist) were greater ($p < 0.05$) in the SHRHNa compared to those obtained in the SHRNNa group. Moreover, in both experimental groups, these responses were attenuated ($p < 0.05$) by both low and high doses of 5-MeU (Table 3).

Renal Excretory Response in SHR with Normal and High Sodium Intake

In the control phase, V , $U_{Na}V$ and FE_{Na} were greater in SHRHNa compared to those in SHRNNa-

Na. In both SHRHNa and SHRNNa groups, during the second phase, close renal arterial infusion of PE caused significant reductions in V , $U_{Na}V$ and FE_{Na} of comparable magnitude when compared to the corresponding values obtained in the vehicle treated first phase (all $P < 0.05$). However, this close renal arterial infusion of PE did not change MAP or RAP in either group (Table 4). Infusion of PE reduced the glomerular filtration rate (GFR) in both experimental groups but these reductions did not attain statistical significance in either group (Table 4). It was also observed that PE infusion reduced RCP in both experimental groups but only became significant ($P < 0.05$) in the SHRHNa group (Table 4). The magnitudes of the reductions in V , $U_{Na}V$ and FE_{Na}

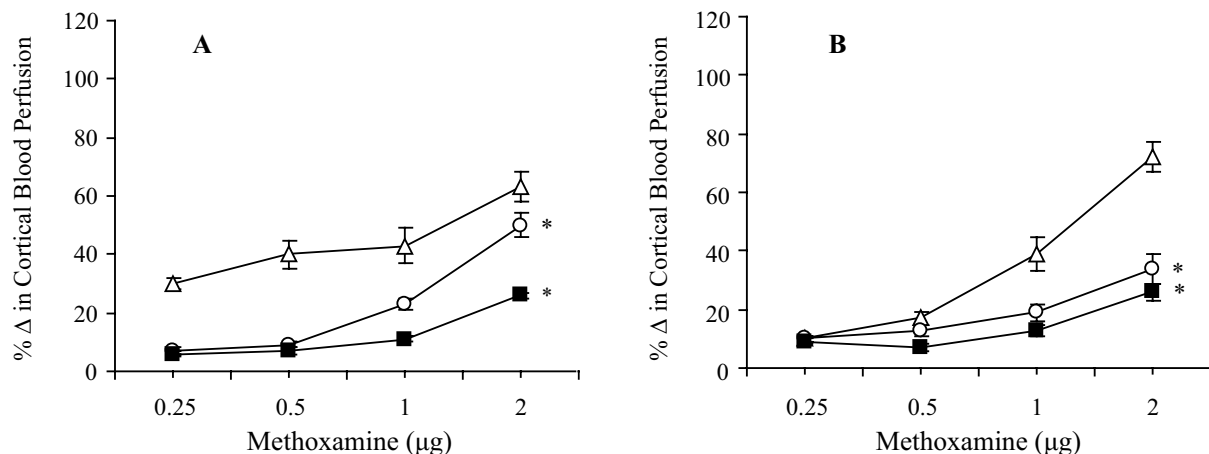


Fig. 3. Methoxamine induced renal cortical vasoconstrictor responses in SHR with high (A) and normal (B) sodium intake in the presence and absence of 5-methylurapidil (Δ Saline, $\circ$ low dose and $\blacksquare$ high dose). *, $P < 0.05$ vs. Saline, ($n = 6$)

Ryc. 3. Korowa odpowiedź zwężająca naczynia nerek wywołana metoksaminą u szczurów z dużym ($n = 6$) i normalnym ($n = 6$) spożyciem sodu w obecności i braku 5-metylurapidilu (Δ sól fizjologiczna, $\circ$ mała dawka i $\blacksquare$ duża dawka). * $P < 0,05$ vs sól fizjologiczna ($n = 6$)

Table 4. Renal hemodynamic and excretory functional responses to phenylephrine (PE) in the absence and presence of 5-methylurapidil in SHR treated with normal ($n = 6$) or high ($n = 6$) sodium diet. *, $P < 0.05$ vs control phase; **, $P < 0.05$ vs control phase. *BPU = blood perfusion unit.

Tabela 4. Odpowiedź hemodynamiczna i wydalinicza nerek na fenyloefrynę (PE) w obecności lub braku 5metylurapidilu u szczurów, którym podawano normalna ($n = 6$) lub dużą ($n = 6$) ilość sodu w diecie. * $p < 0,05$ vs fazy kontrolnej, ** $p < 0,05$ vs fazy kontrolnej, #BPU – jednostka perfuzji krwi

Parameters (Wskaźniki)	SHR with normal Na intake (Szczury, którym podawano normalną ilość Na)			SHR with high Na intake (Szczury, którym podawano dużą ilość Na)		
	control	PE (100 µg/kg/h)	PE (100 µg/kg/h) +5-MeU (10 µg/kg/h)	control	PE (100 µg/ kg/h)	PE (100 µg/kg/h) +5-MeU (10 µg/ kg/h)
MAP (mm Hg)	135 ± 3	136 ± 2	127 ± 8	143 ± 3	137 ± 1	133 ± 3
RAP (mm Hg)	150 ± 6	152 ± 6	137 ± 11	136 ± 4	131 ± 4	132 ± 4
RCP (#BPU)	231 ± 41	207 ± 38	234 ± 33	225 ± 25	160 ± 13*	143 ± 6**
GFR (ml/Kg/min)	2.4 ± 2	1.8 ± 1	3.0 ± 2	1.6 ± 0.3	1.3 ± 1	2.4 ± 1
V (µl/min/Kg)	150 ± 40	101 ± 21*	217 ± 21	204 ± 44	69 ± 18*	232 ± 20**
UNaV (µM/min/Kg)	30 ± 5	25 ± 3	38 ± 4	40 ± 6	8 ± 2*	35 ± 2**
FENa (%)	6 ± 2	1 ± 0.4	5 ± 3	10 ± 2	2 ± 1*	8 ± 1

in response to PE in the second phase in SHRHNa were greater compared to those in the SHRNNa group (Table 4). During the third phase of the experiment, PE was infused in the presence of a concomitant infusion of 5-MeU close renal arterially. It was observed that 5-MeU significantly abolished (all $P < 0.05$) the antinatriuretic and antidiuretic effects of PE in the SHRHNa (Table 4) and V, $U_{Na}V$, FE_{Na} , RCP and GFR returned to the levels nearly similar to the control values (Table 4). However, in the SHRNNa group, the antinatriuretic and antidiuretic responses were ameliorated by 5-MeU to some extent but did not return to control levels (Table 4). It was further observed that 5-MeU and PE infusion

did not have any effect on the MAP and RAP in either experimental group (Table 4).

Discussion

The renal vasoconstrictor and excretory responses to renal nerve stimulation or α_1 -ARs agonist treatment are mediated by α_1 -ARs [18, 19]. A high salt intake has been associated with an augmented responsiveness of the renal α_1 -ARs leading to impaired Na^+ excretion and altered renal vascular resistance which may accelerate the rise in blood pressure in rats with a genetic predisposition

to hypertension [4]. Among the subtypes of renal α_1 -ARs, a major role for α_{1A} -adrenoceptors has been suggested in the regulation of renal hemodynamics *in vivo* [20]. The basis of the present study was to examine the hypothesis that the renal and excretory responses to adrenergic stimulation mediated via α_{1A} -ARs might be enhanced in the SHR subjected to an elevated dietary sodium intake over a six-week period. During these six-weeks of the high salt treatment regime, SHR showed significant increases in WI, V and $U_{Na}V$. These changes reflect a normal physiological response of the body to the high salt load.

In the present study, we have studied the sensitivity of the cortical vasculature to adrenergic agonists, as the blood flow in the cortex constitutes ~90% of the total renal blood flow, and hence plays the primary role in the regulation of renal hemodynamics.

Perhaps, the most interesting finding of the study is, that independent of any further increase in arterial pressure, the high salt load in the SHR increased the sensitivity of the renal cortical vasculature to intrarenally administered adrenergic agonists viz. NA, PE, with maximum pressor response seen for ME, compared to SHR fed a normal diet. Thus, in the present study it appeared that in the SHR, high sodium intake for six weeks increased the renal cortical smooth muscle responsiveness to the vasoconstrictor stimuli, more particularly to ME, that acts on α_{1A} -adrenoceptors.

This enhanced vascular smooth muscle responsiveness may lead to a mechanism where vessels provide a greater resistance to blood perfusion resulting in a higher pressure which then predisposes to an increase in arterial wall thickness and remodeling mechanisms [21]. Further, this increase in the vascular resistance due to high sodium intake indicates that an increase in peripheral vascular resistance may predispose any individual to salt-induced blood pressure response. Indeed, a similar relationship between the effect of high sodium intake and changes in vascular smooth muscle reactivity has been reported in humans with hypertension which is etiologically related to high salt intake. In these individuals, hypertension is found to be mediated through enhanced forearm vascular resistance, leading to vasoconstriction and further elevation of total peripheral vascular resistance [7].

A potential reason underlying the increase in vascular reactivity that occurs after sodium loading might be due to sodium-dependent impairment of noradrenaline uptake in SHR [22]. The present study showed that, independent of any change in blood pressure, enhanced renal cortical vascular sensitivity after high sodium intake in SHR might be due to the involvement of a renal

vascular α_1 -adrenergic mechanism. It is suggested that this mechanism might be one of the factors in the salt-related increase in the renal vascular resistance in SHR on high sodium intake. Indeed, previous *in vitro* studies have demonstrated that after exposure to a high sodium diet there was a significant elevation in renal α_1 -AR density [3, 4, 23]. In the present study, a moderate salt load in the SHR caused functional alterations in the renal cortical α_1 -AR density, which was shown as enhanced sensitivity of the renal cortical vasculature to α_1 -AR agonists. This was particularly evident in the case of ME (which is a specific α_{1A} -adrenergic agonist) and thus led us to suggest that, among the subtypes of α_1 -AR, α_{1A} -AR might be responsible for the observed enhanced sensitivity. Moreover, the adrenergically induced renal cortical vascular responses to exogenously administered adrenergic agonists were attenuated by both low and high doses of 5-MeU in SHRHNa and SHRNNa. These results further supported the functional involvement of α_{1A} -AR in adrenergically induced renal cortical vasoconstriction in SHR regardless of dietary sodium changes. Studies have also shown that gene expression of α_{1A} -AR along with elevated blood pressure and sympathetic activity is observed in SHR when compared to WKY [15].

In the present study, it was also observed that the excretory responses to PE obtained in the vehicle treated control phase were greater in SHRHNa compared to SHRNNa. PE infusion led to significant reductions in V, $U_{Na}V$ and FE_{Na} compared to the vehicle treated control phase in both SHRNNa and SHRHNa rats, thus strengthening the fact that activation of α_1 -AR leads to antidiuresis and antinatriuresis in SHR [24]. However, the glomerular filtration rate remained relatively unchanged during the infusion of phenylephrine, while PE infusion in the second phase caused a marked decrease in RCP of SHRHNa. The observed antinatriuretic and antidiuretic response to PE in the present study was more pronounced in SHRHNa compared to SHRNNa which may suggest an augmented α_1 -adrenergic activity at the tubular level and this could be due to the high salt load in these rats. Moreover, the attenuation of antinatriuretic and antidiuretic responses to PE by 5-MeU in SHRHNa but not in SHRNNa supports the involvement of a α_{1A} -adrenergic receptor in the antinatriuresis and antidiuresis at the tubular level. Further studies are required to investigate the possible impact of high dietary sodium on the functionality of other subtypes of α_1 -AR present in the kidney of the SHR.

In conclusion, our study suggested that both at the renal cortical vascular and tubular functional level the activity of α_1 -AR adrenergic receptor re-

sponsiveness were increased by the high salt load in SHRHN_a as compared to SHRNN_a. Among the subtypes of α_1 -AR adrenergic receptors, α_{1A} -AR seems to play a major role in the observed renal cortical vascular supersensitivity, augmented an-

tidiuresis and antinatriuresis in SHRHN_a. The data also suggest that irrespective of dietary sodium changes α_{1A} -ARs are the functional subtypes involved in the regulation of adrenergically induced renal cortical vasoconstriction in SHR.

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