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Role of γ melanocyte-stimulating hormone–renal melanocortin 3 receptor system in blood pressure regulation in salt-resistant and salt-sensitive rats

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Abstract

Melanocortin 3 receptor (MC3-R) has high affinity and specificity to γ melanocyte-stimulating hormone (γMSH), a natriuretic peptide involved in regulation of blood pressure (BP) and sodium excretion. Recent studies showing increased MC3-R expression and elevated plasma γMSH in normal rats fed a high-salt diet support the role of this system in sodium homeostasis. We hypothesized that dysregulation of MC3-R response to dietary salt may contribute to salt retention and BP elevation in salt-sensitive hypertension. We examined renal MC3-R expression, plasma γMSH concentration, and response to MC3-R agonist and antagonist in Dahl salt-sensitive (DSS) and Dahl salt-resistant (DSR) rats fed high-salt (8%) or low-salt (0.07%) diets for 3 weeks. Consumption of high-salt diet significantly increased BP in the DSS but not the DSR group. High-salt diet led to a 5-fold increase in plasma γMSH and a 2-fold increase in renal MC3-R in DSR rats. Plasma γMSH and renal MC3-R abundance in DSS rats were maximally elevated on low-salt diet and remained unchanged on high-salt diet. Administration of MC3-R agonist melanotan II significantly lowered BP and raised fractional Na excretion in the DSR but not the DSS rats consuming high-salt diet. In contrast, MC3-R antagonist SHU9119 significantly raised BP and lowered fractional Na excretion in both groups. Thus, the data suggest that γMSH–renal MC3-R pathway is activated and appears to be biologically functional in the DSS rats.

1. Introduction

Several subtypes of melanocortin receptors have thus far been identified in humans [1]. Among them, melanocortin 3 receptor (MC3-R) has a higher affinity and specificity to γ melanocyte-stimulating hormone (γMSH) [2-4], a natriuretic peptide [5-10] that is synthesized from the prohormone proopiomelanocortin (POMC) in the intermediate lobe of the pituitary gland [11]. Melanocortin 3 receptor is found in diverse tissues such as bone, brain, joint, adrenal gland, heart, and kidney [12,13]. There is abundant physiologic evidence that binding of γMSH to MC3-R in the kidney can promote natriuresis in rodents [14]. Consumption of high-salt diet for 2 to 3 weeks up-regulates pituitary POMC messenger RNA (mRNA) and protein expression, raises plasma γMSH, and increases urinary sodium excretion without changing glomerular filtration rate in normal rats [11]. In addition, high salt intake up-regulates expression of prohormone convertase 2 that cleaves POMC into the active peptide γMSH [15]. The relevance of γMSH to regulation of arterial pressure and sodium homeostasis is supported by the observation that deletion of the prohormone convertase 2 gene results in salt-sensitive hypertension in mice [16].

Melanocortin 3 receptor is a G protein–coupled membrane receptor that acts in line with cyclic adenosine monophosphate activation [17-19] in various tissues. There is evidence supporting the role of γMSH and its receptors in the pathogenesis of obesity, cardiovascular disorders, and hypertension. Ni et al [20] reported up-regulation of MC3-R expression in the inner medullary region of the kidney in rats fed a high-salt diet. This was associated with increased urinary excretion of cyclic adenosine monophosphate, pointing to heightened receptor activity with high-salt diet. The authors

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further showed a significant increase in MC3-R mRNA expression in isolated inner medullary collecting duct cells exposed to a high-salt medium. This was accompanied by a dose-dependent response to addition of γMSH to the incubation media [20]. High-salt diet has been shown to result in significant rise in arterial pressure in MC3-R knockout mice but not in the wild-type mice [16]. Taken together, these observations substantiate the role of γMSH and MC3-R system in the sodium homeostasis. It is, therefore, plausible that impaired γMSH–MC3-R system may be involved in the pathogenesis of hereditary forms of salt-sensitive hypertension. Melanocortin system and the related receptors could potentially participate in the development of salt-sensitive hypertension independently or as an intermediate modulator in conjunction with other neurohumoral pathways involved in regulation of renal salt handling.

Dahl salt-sensitive (DSS) rats are commonly used as a prototypical model of salt-sensitive hypertension. In an earlier study, Hao and Rabkin [21] found that consumption of high-salt diet increases POMC mRNA abundance in the pituitary glands of Dahl salt-resistant (DSR) but not DSS rats. In another study, Mayan et al (Abstract: *Hypertension* 1993;22:417) showed that, unlike normal rats, DSS rats fail to increase either pituitary POMC mRNA expression or plasma MSH concentration when fed a high-salt diet. To our knowledge, the effect of dietary salt intake on renal MC3-R expression in DSS rat has not been previously investigated. The present study was designed to test the hypothesis that impaired renal sodium handling and the consequent rise in arterial pressure with high salt intake in DSS rats may be associated with and, in part, mediated by dysregulation of γMSH–renal MC3-R axis. The study revealed that the γMSH–MC3-R pathway is activated and appears to be biologically functional in the DSS rats regardless of dietary salt intake.

2. Materials and methods

2.1. Animals

Experiments were performed using 20-week–old inbred lines of male Dahl SR/JrHsd (SRR) and Dahl SS/JrHsd (SSR) rats maintained as inbred colonies (Harlan, Indianapolis, IN). The Charles Drew University Animal Care and Use Committee approved all experimental protocols. The rats were maintained on tap water and regular diet ad libitum until switched to low-salt (0.07%) or high-salt (8%) diets (Harlan Teklad, Madison WI). Six rats were used in each of the 4 subgroups. The diets were continued for 3 weeks. Blood pressure (BP) and body weight were measured twice weekly. Twenty-four–hour urine collections were obtained using metabolic cages twice before and after initiation of the assigned diets. At the conclusion of the study period, under general anesthesia (using 0.3% of isoflurane inhalation), animals were killed by decapitation. Blood samples were collected, and kidneys were immediately harvested. Kidneys were snap-frozen in liquid nitrogen and stored at −80°C until processed. Another set of 4 rats from each group had the telemetry catheter and transmitter placement to monitor the BP changes before and after the pharmaceutical interventions with MC3-R/MC4-R agonist and antagonist. Blood and urine samples were collected to measure the fractional excretion of sodium immediately before and 2 to 6 hours after the intervention.

2.2. Arterial BP determination by telemetry

Rat BP transmitter (Data Sciences International, Saint Paul, MN) was used to directly measure arterial pressure. The rats were anesthetized with isoflurane and kept on a heated pad throughout the surgery and recovery periods to maintain body temperature. Abdominal hair was shaved, and a midline incision was made to access the abdominal aorta. The catheter was inserted into the abdominal aorta, and the transmitter probe was positioned subcutaneously on the right side of the abdominal wall. Rats were allowed a 7-day recovery period before the initiation of the study. Data were acquired and analyzed using an acquisition program (Dataquest ART Version 3.1, Data Sciences International). Blood pressure data were sampled for 4 hours daily as well as before and after pharmacologic interventions.

2.3. Response to MC3-R/MC4-R agonist and antagonist

In a separate set of experiments, the animals were treated with intraperitoneal injection of 1 mg/kg melanotan II (MT II, MC3-R/MC4-R agonist; Phoenix Pharmaceuticals, Belmont, CA) and 100 μg/kg SHU9119 (MC3-R/MC4-R antagonist, Phoenix Pharmaceuticals) in DSR and DSS rats that were fed a high-salt diet. Arterial pressure was measured, and urine and plasma were collected at baseline and after administration of MT II and SHU9119. Fractional excretion of sodium was calculated using the standard formula.

2.4. Serum creatinine and urine protein measurements

Serum creatinine was determined by DT-II chemistry analyzer (Ortho Clinical Diagnostics, Raritan, NJ). Urine protein and creatinine concentrations were measured by RANDIL Chemistry Laboratory (Michigan, PA).

2.5. Blood pressure measurement by tail-cuff method

Collection of BP data was begun after a week of conditioning. Conscious rats were placed in a restrainer on a warming pad and allowed to rest inside the cage for 15 minutes before BP measurements. Rat tails were placed inside a tail cuff, and the cuff was inflated and released several times to allow the animal to be conditioned to the procedure. Three consecutive BP readings were taken by a rat tail BP monitor attached to a student oscillograph (Harvard Apparatus, Holliston, MA) and averaged for presentation.

2.6. Radioimmunoassay

Frozen plasma samples were thawed on ice, extracted, and eluted through Sep-Column Chromatography cartridges

purchased from Peninsula Laboratories, Belmont, CA. The eluate was lyophilized and stored at −70°C until assayed by a commercially available radioimmunoassay kit (Peninsula Laboratories) with ^{125}I-labeled γ^2MSH as a tracer. The antibody used is highly sensitive for γ^2MSH and has little to no cross-reactivity with α or βMSH. The assay was carried out according to the manufacturer's instructions. The γMSH level was measured in the plasma samples after standardizing and calculating the radioactivity of the γMSH using a 300 SL automatic Triple to Double Coincidence Ratio liquid scintillation counter (Hidex, Personal Life Science, Turku, Finland).

2.7. Tissue preparation

Kidney tissue was homogenized in 3 mL of lysis buffer at 0°C to 4°C using a Polytron tissue mixing and blending device (Brinkmann Instruments, Westbury, NY). Homogenates were centrifuged at 12 000*g* for 10 minutes at 4°C to precipitate tissue debris. The supernatant was used to perform the Western blot analyses. Total protein concentration was determined with the use of a Bio-Rad kit (Bio-Rad Laboratories, Hercules, CA).

2.8. Western blot analyses

Western blot analysis was performed using an antiserum raised against rat MC3-R (Santa Cruz Biotechnology, Temecula, CA) at a 1:800 dilution. Bound antibody was visualized using horseradish peroxide–conjugated donkey anti-rabbit secondary antibody diluted 1:1000 for 30 minutes (RPN 2108 ECL kit; Amersham, Arlington Heights, IL); the chemiluminescent signal was detected on x-ray film and quantitated by densitometric scanning using a densitometer. Data were normalized against glyceraldehyde-3-phosphate dehydrogenase that was used as the housekeeping protein.

2.9. Statistical analysis

Student *t* test and 1-way analysis of variance were used in statistical analysis of the data as appropriate. Results are expressed as the mean ± standard error. *P* values less than .05 were considered significant.

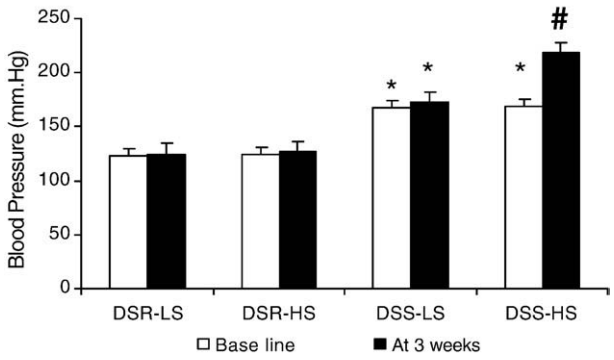

Fig. 1. Blood pressure measurements at baseline and 3 weeks after consumption of a low-salt (0.3%) or high-salt (8%) diet in DSR and DSS rats. n = 6 animals in each group. *P < .01 vs DSR rats and DSS-HS at 3 weeks; #P < .001 vs all other groups. HS indicates high salt; LS, low salt.

Table 1
The body weight, creatinine clearance, and urinary protein excretion of DSR and DSS rats receiving high-salt (8%) or low-salt (0.3%) diets for 3 weeks

	DSR-LS[a]	DSR-HS[a]	DSS-LS[a]	DSS-HS[a]
Creatinine clearance (mL/[min 100 g body weight])	0.25 ± 0.0	0.46 ± 0.1†	0.11 ± 0.01*	0.10 ± .02*
Urine protein excretion (mg/mg creatinine)	3.6 ± 0.2	5 ± 0.5	13 ± 1.9*	79 ± 18*,†

LS indicates low salt; HS, high salt.
[a] n = 6 animals.
* Salt sensitive vs salt resistant, $P \leq .05$.
† High vs low salt, $P \leq .05$.

3. Results

3.1. General data

Consumption of high-salt diet resulted in a marked rise in arterial pressure in the DSS rats but had no significant effect on arterial pressure in DSR animals (Fig. 1). The rise in arterial pressure in response to high-salt diet in DSS rats found by the tail-cuff method was confirmed by telemetry in another set of animals. Creatinine clearance was significantly lower in DSS compared with DSR rats consuming low-salt diet. Consumption of high-salt diet resulted in a significant increase in creatinine clearance in DSR but not in DSS rats. Urinary protein excretion was significantly higher in DSS than in DSR rats consuming low-salt diet. Consumption of high-salt diet resulted in a significant rise in urinary protein excretion in DSS but not in DSR rats (Table 1).

3.2. Plasma γMSH data

Data are shown in Fig. 2. Plasma γMSH level was significantly elevated in the DSS rats consuming low-salt diet compared with that found in the corresponding DSR rats (16.8 ± 3.4 vs 3.1 ± 1.4 pg/mL, P < .05). Consumption of the high-salt diet resulted in a more than a 5-fold increase in circulating γMSH in the DSR group (3.1 ± 1.4 vs 19.9 ± 1.7

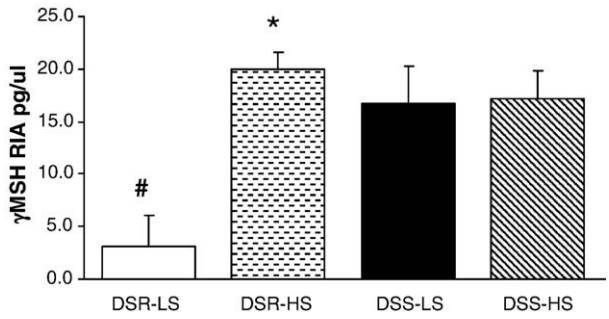

Fig. 2. Plasma γMSH concentration in DSR and DSS rats fed a low- or high-salt diet for 3 weeks. n = 6 animal in each group. *P < .001, DSR-HS vs DSR-LS; #P < .05, DSR-LS vs all other groups.

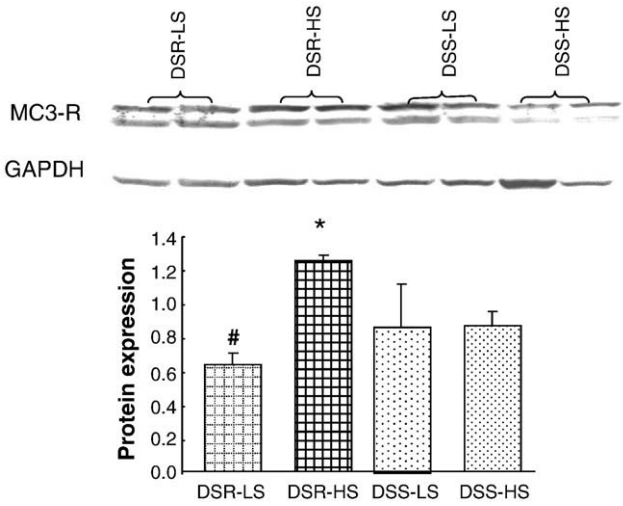

Fig. 3. Representative Western blot depicting MC3-R protein abundance in the kidney of DSR and DSS rats fed a low- or high-salt diet for 3 weeks. n = 6 animals in each group. *$P < .05$, DSR-HS vs DSR-LS; $^{\#}P < .05$, DSR-LS vs all other groups.

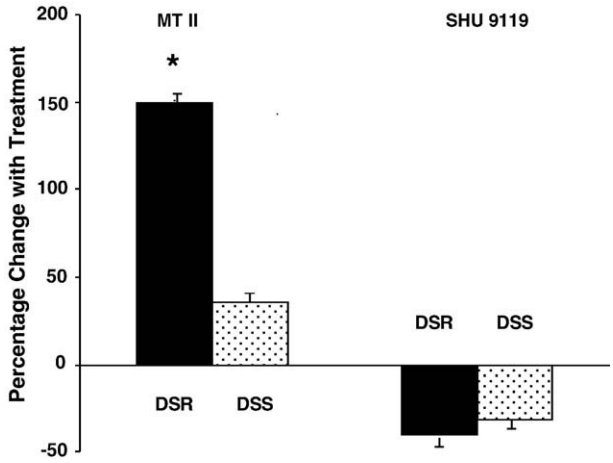

Fig. 5. Comparison of percentage change in fractional excretion of sodium in response to intraperitoneal administration of MC3-R agonist MT II and antagonist SHU9119 between DSR and DSS rats fed a high-salt (8%) diet for 3 weeks. *$P < .05$, DSR vs DSS.

pg/mL, $P = <.01$). Plasma γMSH level was significantly elevated in the DSS rats consuming low-salt diet and failed to rise further in response to high salt intake (16.8 ± 3.4 vs 17.6 ± 2.6 pg/mL, not significant).

3.3. Renal MC3-R protein expression

Data are shown in Fig. 3. The kidney tissue MC3-R protein abundance was significantly lower in DSR compared with DSS rats on low-salt diet. Consumption of high-salt diet resulted in a 2-fold increase in kidney tissue MC3-R protein abundance in the DSR rats. In contrast, high-salt diet failed to significantly change MC3-R protein abundance in the kidneys of DSS rats.

3.4. Response to MC3-R/MC4-R agonist (MT II) and antagonist (SHU9119)

Data are shown in Figs. 4 and 5. Administration of MC3-R/MC4-R agonist MT II led to a significant fall in arterial pressure and a significant rise in fractional excretion of sodium in DSR rats consuming high-salt diet. In contrast, MT II administration had no significant effect on arterial pressure and had minimal impact on fractional excretion of sodium in the corresponding DSS group. Administration of MC3-R/MC4-R antagonist SHU9119 resulted in a significant rise in arterial pressure and a significant fall in fractional excretion of sodium in both DSR and DSS groups. It should be noted that impairment of renal function could have contributed to the poor natriuretic response to administration of MC3-R agonist in the DSS rats consuming high-salt diet.

4. Discussion

γ Melanocyte-stimulating hormone is a natriuretic peptide [5-10] that plays a role in sodium homeostasis [11,22] via activation of MC3-R. High-salt diet has been shown to significantly raise arterial pressure in MC3-R

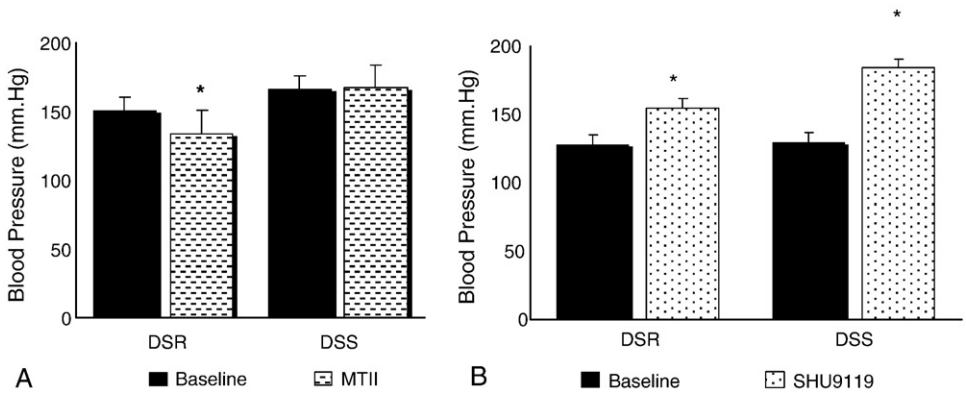

Fig. 4. Blood pressure response to intraperitoneal administration of MC3 agonist MT II and antagonist SHU9119 in DSR and DSS rats fed a high-salt (8%) diet for 3 weeks. *$P < .01$ vs untreated group.

knockout mice but not in the wild-type mice [16]. These observations have provided convincing evidence for the role of MC3-R in regulation of BP and its deficiency as a potential cause of salt-sensitive hypertension. Therefore, the present study was undertaken to discern whether dysregulation of γMCH–renal MC3-R system in response to alterations of dietary salt intake is involved in the pathogenesis of hypertension in DSS rats fed a high-salt diet.

As expected, consumption of the high-salt diet resulted in a marked rise in arterial pressure in our salt-sensitive, but not salt-resistant, rats. Consumption of the high-salt diet resulted in significant up-regulation of MC3-R protein abundance in the kidneys of salt-resistant animals. These observations are consistent with the result of the study published by Ni et al [20] who found marked up-regulation of renal tissue MC3-R expression in normal rats fed a high-salt diet. Up-regulation of renal tissue MC3-R expression with high-salt diet in the salt-resistant animals was coupled with marked elevation of plasma γMSH concentration. In contrast to the salt-resistant animals, plasma γMSH concentration and kidney tissue MC3-R abundance were markedly elevated in salt-sensitive animals on low-salt diet and failed to rise further on high-salt diet. These observations suggest that γMSH–MC3-R pathway is activated in the DSS rats regardless of dietary salt intake.

In an attempt to discern the contribution of γMSH–renal MC3-R pathway in regulation of BP and renal sodium handling in salt-resistant and salt-sensitive animals, we next explored the impact of pharmacologic activation and blockade of the MC3-R. Administration of MC3-R/MC4-R agonist MT II led to a significant fall in arterial pressure and a significant rise in fractional excretion of sodium in DSR rats consuming high-salt diet. In contrast, MT II administration had no significant effect on arterial pressure and had minimal impact on fractional excretion of sodium in the corresponding DSS group. Administration of MC3-R antagonist SHU9119 resulted in a significant rise in arterial pressure and a significant fall in fractional excretion of sodium in both DSR and DSS groups. This observation illustrates the physiologic role of γ MSH–renal MC3-R system in both DSR and DSS. Moreover, the observed elevation of plasma γMSH and renal tissue MC3-R in moderately hypertensive DSS rats consuming low-salt diet suggests that hypertension per se, independent of dietary salt intake, can potentially stimulate the γMSH–renal MC3-R pathway. This phenomenon may represent a physiologic response aimed at attenuating the severity of hypertension. It should be noted that renal function was impaired in the salt-sensitive animals. The effect of impaired renal function on γMSH–renal MC3-R system in the salt-sensitive animals is uncertain and requires further investigation.

In conclusion, the data presented in this study confirm the contribution of γMSH–renal MC3-R pathway to regulation of arterial pressure and sodium homeostasis in both salt-resistant and salt-sensitive animals. The data further suggest that γMSH–renal MC3-R pathway is activated irrespective of dietary salt intake in the DSS rats. This viewpoint is supported by marked and equal elevation of plasma γMSH and renal tissue MC3-R expression on both low- and high-salt diets, lack of hypotensive response to MC3-R agonist, and significant hypertensive response to MC3-R antagonist in salt-sensitive animals.

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