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Overexpression of HIF-1α transgene in the renal medulla attenuated salt sensitive hypertension in Dahl S rats

Qing Zhu, Zhengchao Wang [1], Min Xia, Pin-Lan Li, Fan Zhang, Ningjun Li *

Department of Pharmacology & Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298

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ABSTRACT

Hypoxia inducible factor (HIF)-1α-mediated gene activation in the renal medulla in response to high salt intake plays an important role in the control of salt sensitivity of blood pressure. High salt-induced activation of HIF-1α in the renal medulla is blunted in Dahl S rats. The present study determined whether the impairment of the renal medullary HIF-1α pathway was responsible for salt sensitive hypertension in Dahl S rats. Renal medullary HIF-1α levels were induced by either transfection of HIF-1α expression plasmid or chronic infusion of $CoCl_2$ into the renal medulla, which was accompanied by increased expressions of anti-hypertensive genes, cyclooxygenase-2 and heme oxygenase-1. Overexpression of HIF-1α transgenes in the renal medulla enhanced the pressure natriuresis, promoted the sodium excretion and reduced sodium retention after salt overload. As a result, hypertension induced by 2-week high salt was significantly attenuated in rats treated with HIF-1α plasmid or $CoCl_2$. These results suggest that an abnormal HIF-1α in the renal medulla may represent a novel mechanism mediating salt-sensitive hypertension in Dahl S rats and that induction of HIF-1α levels in the renal medulla could be a therapeutic approach for the treatment of salt-sensitive hypertension.

1. Introduction

Salt-sensitive hypertension accounts for 50% of hypertensive population [1,2]. Importantly, the salt sensitivity of blood pressure is closely associated with a much greater propensity to develop organ injuries in hypertension [2–4]. Mechanism for salt-sensitive hypertension is not fully understood. It is well documented that renal medullary function play an important role in the regulation of renal sodium excretion and arterial blood pressure, and that dysfunction in the renal medulla is involved in salt-sensitive hypertension [5–10]. We have recently demonstrated that the transcription factor hypoxia inducible factor (HIF)-1α-mediated gene activation in the renal medulla is an important adaptive mechanism in response to high salt intake, which leads to inductions of various protective factors in the renal medulla and promotes extra sodium excretion [11].

HIF-1α and some of its target genes, such as nitric oxide synthase (NOS), cyclooxygenase-2 (COX-2) and hemeoxygenase-1 (HO-1), are highly expressed in the renal medulla [6,10,12–15]. These HIF-1α target genes in the renal medulla are up-regulated in response to high salt intake [6,13–16]. The products of these genes play critical roles in regulating renal medullary blood flow and tubular activity, and thereby maintaining the constancy of body fluid volume and arterial blood pressure [6,10,13,14,17–19]. Interestingly, inhibition of these genes and/or the enzymes encoded by these genes within the renal medulla reduces sodium excretion and increases salt sensitivity of arterial blood pressure [6,10,13,14,17–19]. We previously showed that high salt intake increased HIF-1α levels in the renal medulla [11], and that inhibition of HIF-1α blocked the activation of its target genes in the renal medulla in response to high salt intake and promoted sodium retention, consequently producing salt-sensitive hypertension [11]. This previous study was carried out in normotensive animals and suggested that HIF-1α-mediated gene regulation in the renal medulla represents an important molecular adaptive mechanism in response to high salt intake and plays a crucial role in the maintenance of sodium balance. However, it remains unknown whether renal medullary HIF-1α pathway is involved in the development of hypertension in salt-sensitive individuals.

Dahl salt sensitive hypertensive rat is a widely used genetic model of human salt-sensitive hypertension that exhibits many phenotypic characteristics in common with human hypertension [3,20–23]. Renal medullary dysfunction is one of the major mechanisms for this rat strain to develop hypertension [7–10]. Most interestingly, the above protective genes regulated by HIF-1α has been shown to be impaired this animal model and deficiencies of these HIF-1α target genes in the renal medulla are considered to be responsible for the development of hypertension in Dahl S rats [9,10,24–27]. We recently showed that upregulation of HIF-1α levels in response to high salt intake was blunted in the renal medulla in Dahl S rats [28]. We

* Corresponding author at: Department of Pharmacology & Toxicology, Medical College of Virginia Campus, Virginia Commonwealth University, P.O. Box 980613, Richmond, VA 23298. Tel.: +1 804 828 2071; fax: +1 804 828 4794.
E-mail address: nli@vcu.edu (N. Li).
[1] Co-first author.

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therefore hypothesized the abnormal responses of the above protective genes are due to a defect in renal medullary HIF-1α and that impairment in HIF-1α-mediated gene activation in the renal medulla is responsible for salt sensitive hypertension in Dahl S rats. In the present study, we induced the expression of HIF-1α levels in the renal medulla by overexpression of HIF-1α transgenes or infusion of $CoCl_2$, a HIF-1α inducer, into the renal medulla and then determined the improvement of renal sodium handling and salt-sensitive hypertension in this animal model. Our results suggested that restoration of the deficit in HIF-1α-mediated gene activation in the renal medulla attenuated salt-sensitive hypertension through the improvement of sodium excretion in Dahl S rats.

2. Materials and Methods

2.1. Animal

Experiments were performed in male Dahl S rats (Charles River, Wilmington, MA), weighing 250–350 g. Animals were kept on a low salt diet (0.4%NaCl) and some of them were fed with a high salt diet (4% NaCl) during experiments as indicated in the results section. All animal procedures were approved by the Institutional Animal Care and Use Committee of the Virginia Commonwealth University.

2.2. Transfection of DNA into the renal medulla

Rats were uninephrectomized one week before, and the remaining left kidney was transfected with plasmids encoding human HIF-1α (50 μg) (Addgene, Cambridge, MA) into the renal medulla using *in vivo-jetPEI™* (Polyplus-transfection, New York, NY), a polyethylenimine derivative, in combination with ultrasound radiation as we described before [28]. We used human HIF-1α plasmids because that rat HIF-1α plasmids were not available and that the hypoxia response elements in HIF-1α target genes are conservative among human-rat-mouse [29,30]. We also confirmed that transfection of these human HIF-1α plasmids into cultured rat cells remarkably increased the expression of HIF-1α target genes HO-1 and COX-2 in preliminary experiment. Plasmids expressing luciferase were used in control animals.

2.3. Chronic renal medullary infusion of $CoCl_2$

The rats were uninephrectomized and anesthetized as above. To implant the renal medullary infusion catheter, the left kidney was exposed by a flank incision (1–1.5 cm), and a medullary interstitial catheter (tapered tip, 4-5 mm) was implanted into the kidney. The catheter was made with a number of circular "pig-tail" bends, which prevented the catheter from being pulled out of the kidney during normal movement of the animal. The catheter was anchored into place on the kidney surface with Vetbond Tissue Adhesive (3 M) and a small piece of fat tissue. These catheters were tunneled to the back of neck and connected to an osmotic pump (ALZET, model 2ML2), which contained $CoCl_2$ (2 mmol/L) and was implanted subcutaneously. This technique has been successfully used for chronic infusion into the kidneys previously [31–34]. We also confirmed the successfully chronic infusion using this method by visualizing the delivery of a red dye into the renal medulla and checking no solution left in the pump in preliminary experiments. At the end of experiment, kidneys were removed and rapidly dissected into the renal cortex and medulla and then frozen in liquid N_2. The precise location of interstitial infusion catheter was determined when dissecting kidney tissue. No solution remained in the osmotic pump was also checked and confirmed at the end.

2.4. Measurement of pressure natriuresis in response to the elevations of renal perfusion pressure

Animals were transfected with HIF-1α or control plasmids as described above and maintained on low salt diet. Ten days after transfection, pressure natriuresis studies were performed as described previously [6,35].

2.5. Measurement of urinary sodium excretion in response to acute sodium loading

Additional groups of animals transfected with HIF-1α or control plasmids as above were surgically prepared similar to that in the pressure natriuresis studies and received a continuous infusion of 0.9% NaCl solution containing 2% albumin at a rate of 1 ml/hr/100 g BW throughout the experiment to replace fluid loss. After 1-hour equilibration and two 10-min control period sample collections, a 5% body weight isotonic saline load was administered intravenously and three 10-min samples were collected over 30 minutes [11,36], and then three more 10-min post-control samples were taken. Urinary volume and sodium excretion were measured.

2.6. Measurement of daily sodium balance

Additional groups of animals the same as above were housed in metabolic cages and daily indexes of sodium balance were computed by subtracting urinary sodium excretion from total sodium intake. After 1 day of control measurements, the animals were switched from tap water to 2% NaCl water and experimental measurements were continued for 3 days [37,38].

2.7. Chronic monitoring of arterial blood pressure in conscious rats

A telemetry transmitter (Data Sciences International) was implanted for the measurement of mean arterial blood pressure (MAP) as we described previously [6]. After baseline MAP was recorded on 3 consecutive control days while the rats remained on low salt diet, animals were switched to high salt diet (Dyets, Inc) and MAP was recorded for additional 2 weeks. Four groups of animals, including rats treated with control plasmids (Ctrl) + low salt diet (LS), Ctrl + high salt diet (HS), HIF-1α plasmids + HS and $CoCl_2$ + HS, were examined. At the end of experiment, renal tissues were collected for protein and RNA isolation later.

2.8. Preparation of tissue homogenate and nuclear extracts and Western blot analyses for protein levels of HIF-1α

Renal tissue homogenates and nuclear protein were prepared, and Western blot analyses were performed as described previously [39,40]. Species reactivity of the primary antibody used in the present study included both rat and human (monoclonal, Novus Biologicals, 1:300 dilution). The intensities of the blots were determined using an imaging analysis program (ImageJ, free download from http://rsbweb.nih.gov/ij/).

2.9. RNA extraction and quantitative RT-PCR analysis of heme oxygenase (HO)-1 and cyclooxygenase (COX)-2 mRNA

Total RNA from renal medulla was extracted using TRIzol solution (Life Technologies, Inc. Rockville MD) and then reverse-transcribed (RT) (cDNA Synthesis Kit, Bio-Rad, Hercules, CA). The RT products were amplified using TaqMan Gene Expression Assays kits (Applied Biosystems). The level of 18S ribosomal RNA was used as an endogenous control. The relative gene expressions were calculated in accordance with the ΔΔCt method. Relative mRNA levels were expressed by the values of $2^{-\Delta\Delta Ct}$.

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2.10. Statistics

Data are presented as means ± SE. The significance of differences in mean values within and between multiple groups was evaluated using an ANOVA followed by a Duncan's multiple range test. Student's *t*-test was used to evaluate statistical significance of differences between two groups. $P<0.05$ was considered statistically significant.

3. Results

3.1. Effect of renal medullary transfection of HIF-1α or $CoCl_2$ infusion on the levels of HIF-1α and its target genes in the renal medulla

As shown in Fig. 1, high salt intake failed to significantly upregulate HIF-1α protein levels in the renal medulla in this animal strain, which was consistent with previous report [28]. However, HIF-1α levels were significantly increased in the renal medulla from rats treated with HIF-1α plasmids or $CoCl_2$ after high salt diet (Fig. 1). The mRNA levels of two important HIF-1α target genes, HO-1 and COX2, in the renal medulla were shown in Fig. 2. Similar to the patterns of HIF-1α protein levels, both HO-1 and COX2 transcriptions were remarkably activated in rats treated with HIF-1α plasmids or $CoCl_2$. Although the increases of these HIF-1α target genes in high salt alone group were statistically significant, these responses to high salt intake were marginal and considerably blunted compared with the responses of 3–4 fold increases in normal rats [11]. Treatments with HIF-1α plasmids and $CoCl_2$ recovered the impaired responses of these protective genes transcriptions after high salt challenge. These results verified the successful induction of HIF-1α-mediated gene activation in the renal medulla by HIF-1α plasmids or $CoCl_2$.

3.2. Effects of renal medullary transfection of HIF-1α transgenes on pressure natriuresis in response to the elevations of renal perfusion pressure (RPP)

Both the urine flow and urinary sodium excretion rates were increased in response to the elevation of RPP. However, these pressure diuretic and natriuretic responses were significantly enhanced in HIF-1α plasmids-transfected rats compared with the control group (Fig. 3).

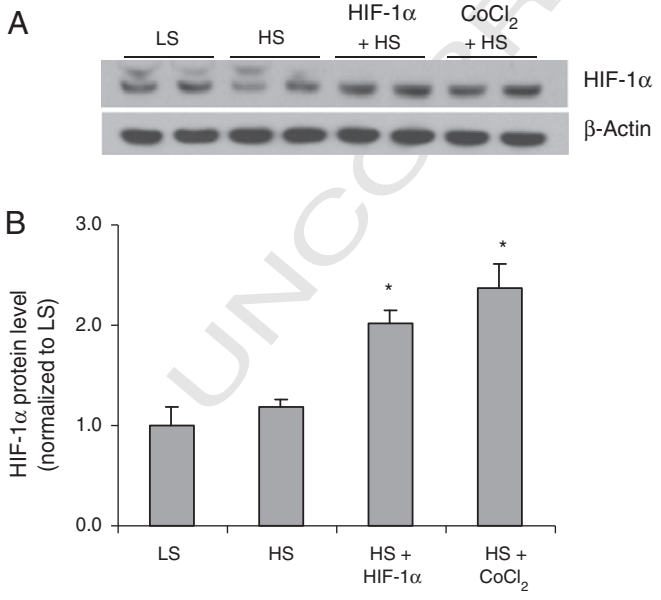

Fig. 1. Effects of renal medullary transfection of HIF-1α transgene or $CoCl_2$ infusion on HIF-1α levels in the renal medulla. A: Representative ECL gel documents of Western blot analyses depicting the protein levels of HIF-1α. B: Summarized intensities of the HIF-1α blots (ratio to β-actin). * $P<0.05$ vs. control ($n=6$). LS = low salt + control vectors, HS = high salt + control vectors, HIF-1α = HIF-1α expression vectors, $CoCl_2$ = $CoCl_2$ infusion.

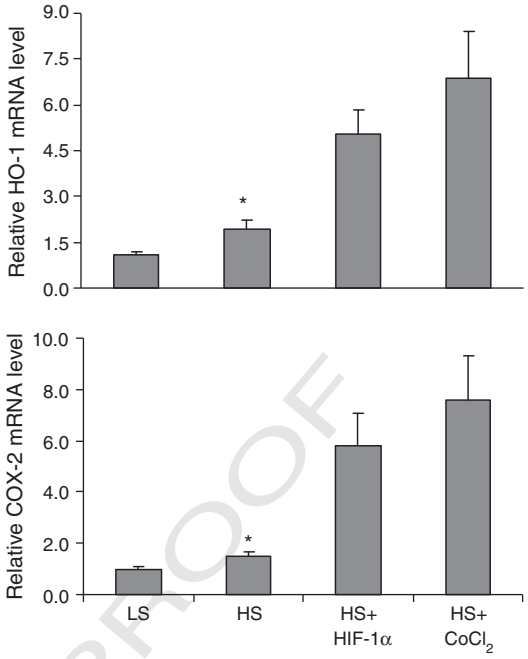

Fig. 2. Effect of HIF-1α transgene overexpression or $CoCl_2$ infusion on the mRNA levels of HIF-1α target genes HO-1 and COX2 in the renal medulla. * $P<0.05$ vs. others including LS group ($n=6$).

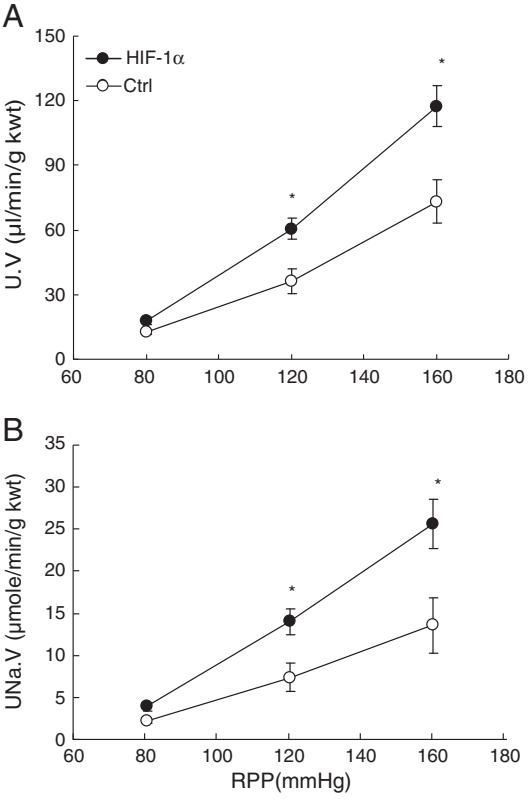

Fig. 3. Effects of renal medullary transfection of HIF-1α transgene on pressure natriuresis. A: urine flow rates (U·V) in response to the elevations of renal perfusion pressure (RPP). B: urinary sodium excretion rates (U_{Na}·V) in response to the elevations of RPP. * $P<0.05$ vs. control ($n=6$).

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3.3. Effects of renal medullary transfection of HIF-1α transgenes on urinary sodium excretion in response to acute sodium loading

Acute sodium loading increased urine volume (U·V) and urinary sodium excretion (U_{Na}·V). These increases in U·V and U_{Na}·V were considerably enhanced in rats treated with HIF-1α plasmids compared with control (Fig. 4).

3.4. Effects of renal medullary transfection of HIF-1α transgeness on salt balance

High salt intake induced a positive daily and cumulative salt balance. The daily positive salt balances were progressively increased in the first two days and decreased on the third day of high salt intake. The high salt-induced positive salt balance was significantly attenuated in rats treated with HIF-1α plasmids compared with control rats (Fig. 5).

3.5. Effects of renal medullary transfection of HIF-1α transgenes or infusion of $CoCl_2$ on arterial blood pressure

There was no difference in baseline mean arterial pressure (MAP) among animas treated with control and HIF-1α plasmids as well as $CoCl_2$ when the animals were fed with a low salt diet. After the rats were challenged with a high salt diet, the MAP were progressively increased from 113 ± 0.9 to 150 ± 7.03 mmHg in control rats. Both treatments of HIF-1α plasmids and $CoCl_2$ remarkably blocked the HS-induced increase in MAP. MAP was only increased to 129 ± 3.1 mmHg in both HIF-1α plasmid-and $CoCl_2$-treated groups by the end of the experiment (Fig. 6).

4. Discussion

The present study demonstrated that induction of HIF-1α-mediated gene activation in the renal medulla stimulated the expression of anti-hypertensive genes in the renal medulla, and consequently enhanced the urinary sodium excretion in response to the elevations of RPP and sodium overloading, reduced sodium retention, as a result, attenuated the salt-sensitive hypertension in Dahl S rats.

Our results showed that local delivery of HIF-1α plasmids or $CoCl_2$ substantially up-regulated the levels of HIF-1α and enhanced the transcription of its target genes in the renal medulla, which validated the manipulation of HIF-1α-mediated gene regulation in the renal medulla and allowed us to evaluate the contribution of HIF-1α-mediated gene activation in the development of hypertension in response to high salt intakes in Dahl S rats.

We first determined the effects of up-regulation in HIF-1α-mediated gene activation on pressure natriuresis. Renal medullary function plays an important role in the regulation of pressure natriuresis [8,41–43] and several HIF-1α target genes such as HO-1, COX-2 and NOS have been reported to be crucial regulators in renal

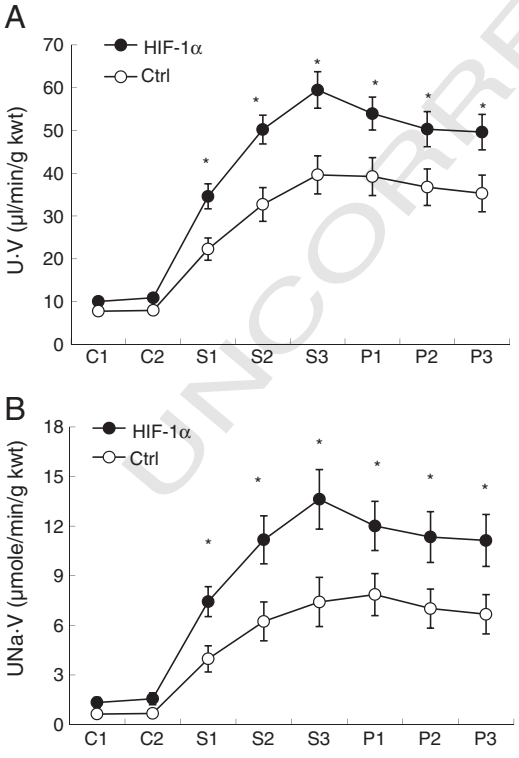

Fig. 4. Effects of renal medullary transfection of HIF-1α transgene on U·V (A) and U_{Na}·V (B) in response to acute Na^+ loading. * P<0.05 vs. control (n = 6). S = sodium loading; P = post sodium loading.

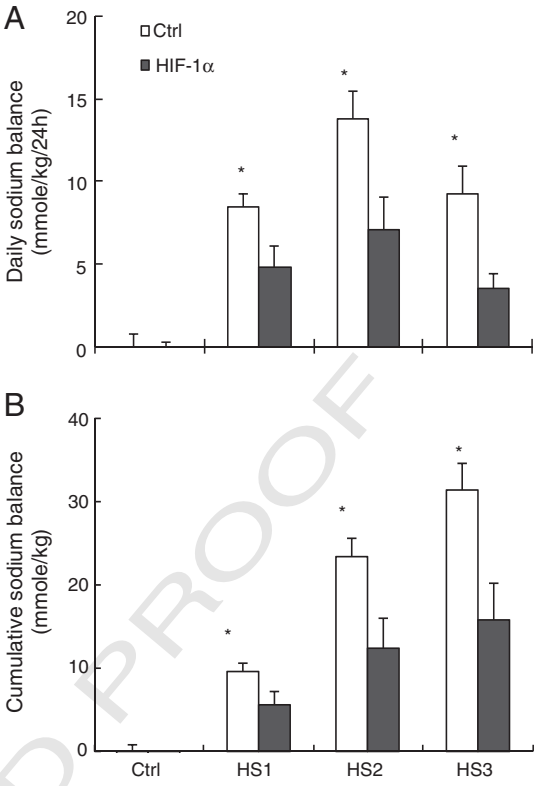

Fig. 5. Effects of renal medullary transfection of HIF-1α transgene on salt balances. A: daily sodium balance. B: cumulative sodium balance. * P<0.05 vs. HIF-1α (n = 6).

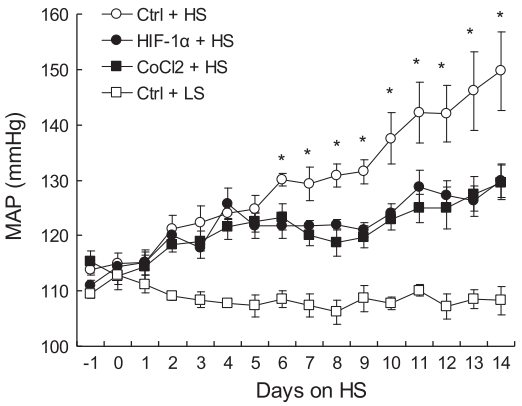

Fig. 6. Effects of renal medullary transfection of HIF-1α transgene or $CoCl_2$ infusion on mean arterial pressure (MAP). * P<0.05 vs. others. (n = 6).

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medullary function and sodium excretion, as well as pressure natriuresis [12,43–46]. Pressure natriuresis has been shown to be significantly blunted in Dahl S rats [47–50]. Interestingly, renal medullary levels of the above enzymes are much lower [9,24,25] and their responses to high salt diet are diminished [9,10,26,27] in Dahl S rats. Increase of HIF-1α levels would be expected to activate the transcriptions of these HIF-1α target genes in the renal medulla, thereby improving pressure natriuresis relationship. Our data showed that transfection of HIF-1α plasmids into the renal medulla significantly enhanced the pressure natriuresis, suggesting that impaired HIF-1α pathway may be responsible for the renal medullary dysfunction in Dahl S rats. Since the products of the enzymes encoded by these HIF-1α target genes have been shown to dilate the medullary vasculature and inhibit the tubular activities [8,41,42,51], the effect of HIF-1α-mediated pathway on pressure natriuresis may be through both vascular and tubular actions.

To further evaluate the impact of renal medullary HIF-1α defect on salt handling, we examined the sodium excretion after acute sodium loading and salt balance after chronic sodium challenge. Because high salt-induced up-regulation of HIF-1α levels in the renal medulla is blunted in Dahl S rats, the impairment in those anti-hypertensive factors, such as COX2 and HO-1, in the renal medulla is probably attributed to the defect in HIF-1α response after high salt challenge in this animal model. Correction of the defect in HIF-1α-mediated gene activation in the renal medulla would improve the salt handling in Dahl S rats. The results from these extra sodium loading experiments demonstrated that restoration of renal medullary HIF-1α levels remarkably improved the capability of the kidneys to remove extra sodium load, which reduced sodium retention. These data additionally suggest that deficiency in renal medullary HIF-1α pathway may contribute to the impaired regulation of sodium excretion in Dahl S rats.

Since pressure-natriuresis and normal renal medullary function are key determinants to the long-term control of arterial blood pressure [7,8,41,42,52], the improvement in sodium excretions in responses to RPP and extra sodium loading would lead to an decrease in MAP in response to high salt intake in Dahl S rats. To test this hypothesis, we compared MAPs between animals transfected with HIF-1α and control plasmids into the renal medulla. It was found that high salt-induced increase in MAP was significantly blocked in HIF-1α plasmids-treated rats. An alternative way to induce HIF-1α by $CoCl_2$ infusion achieved a similar result to block high salt-induced hypertension. It has been shown that high salt-induced activation of HIF-1α-regualted pathways is considered as an adaptive mechanism to high salt intake, which leads to an induction of various protective factors and promotes extra sodium excretion [11]. Therefore, deficiency of HIF-1α-mediated gene transcription in the renal medulla may decrease the production of various protective factors, impair renal medullary function, damage the capability of the kidneys to remove extra sodium load, consequently disrupt salt adaptation and increase the salt sensitivity of arterial blood pressure in Dahl S rats. This deficiency in HIF-1α-mediated gene activation may represent an important mechanism for the development of salt sensitive hypertension. Induction of HIF-1α in the renal medulla may restore the molecular adaptation to high salt intake and stimulate the production of different renal medullary protective or antihypertensive factors, thereby, attenuate salt-sensitive hypertension.

The present study did not attempt to explore the mechanisms that caused the impaired HIF-1α response to high salt in Dahl rats. In this regard, HIF-prolyl hydroxylases, the enzymes that promote the degradation of HIF-1α, may be accountable for it. HIF prolyl-hydroxylases catalyze site-specific proline hydroxylation of HIF-1α and then the hydroxylated HIF-1α is recognized and targeted for degradation by the ubiquitin-proteasome pathway [53,54]. Three isoforms of HIF prolyl-hydroxylase, including prolyl hydroxylase domain-containing proteins 1, 2, and 3 (PHD1, 2, and 3), have been identified [53,55,56]. PHDs are present in the kidneys with PHD2 as the predominant isoform [39,57–60] and PHD2 is most abundantly expressed in the renal medulla [39,60]. It has been shown that high salt-induced inhibition of PHD2 in the renal medulla may be the upstream mediator for the activation of renal medullary HIF-1α in response to high salt challenge [28]. A defect in renal medullary PHD2 could be responsible for the impairment of HIF-1α-mediated salt adaptive pathway in Dahl S rats [28]. Detailed mechanisms associated with renal medullary PHD2 in salt adaptation need to be clarified in future investigations.

In summary, the present study demonstrated that up-regulation of HIF-1α levels in the renal medulla stimulated the transcription of enzymes that produce anti-hypertensive factor in the renal medulla, which corrected the defect in HIF-1α-mediated renal adaptation in response to high salt intake. As a result, this correction improved sodium excretion and attenuated salt-sensitive hypertension in Dahl S rats. It is concluded that deficiency in HIF-1α-mediated gene activation may be responsible for the hypertension in Dahl S rats and correction of this defect may be used to as a therapeutic strategy for salt-sensitive hypertension.

Conflict of Interest

None.

Acknowledgements

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