

## Review Article

# Hypoxia inducible factor-1 $\alpha$ -mediated gene activation in the regulation of renal medullary function and salt sensitivity of blood pressure

Ningjun Li

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

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**Abstract:** Many enzymes that produce natriuretic factors such as nitric oxide synthase (NOS), hemeoxygenase-1 (HO-1) and cyclooxygenase-2 (COX-2) are highly expressed in the renal medulla. These enzymes in the renal medulla are up-regulated in response to high salt intake. Inhibition of these enzymes within the renal medulla reduces sodium excretion and increases salt sensitivity of arterial blood pressure, indicating that these enzymes play important roles in kidney salt handling and renal adaptation to high salt challenge. However, it remains a question what mechanisms mediate the activation of these enzymes in response to high salt challenge in the renal medulla. Interestingly, these enzymes are oxygen sensitive genes and regulated by transcription factor hypoxia-inducible factor (HIF)-1 $\alpha$ . Our recent serial studies have demonstrated that: 1) High salt intake stimulates HIF-1 $\alpha$ -mediated gene expression, such as NOS, HO-1 and COX-2, in the renal medulla, which may augment the production of different antihypertensive factors in the renal medulla, mediating renal adaptation to high salt intake and regulating salt sensitivity of arterial blood pressure. 2) HIF prolyl-hydroxylase 2 (PHD2), an enzyme that promotes the degradation of HIF-1 $\alpha$ , is highly expressed in renal medulla. High salt intake suppresses the expression of PHD2 in the renal medulla, which increases HIF-1 $\alpha$ -mediated gene expressions in the renal medulla, thereby participates in the control of salt sensitivity of blood pressure. 3) The high salt-induced inhibition in PHD2 and the consequent activation of HIF-1 $\alpha$  in the renal medulla is not observed in Dahl salt sensitive hypertensive (Dahl/ss) rats. Correction of these defects in PHD2/HIF-1 $\alpha$ -associated molecular adaptation in the renal medulla improves sodium excretion, reduces sodium retention and attenuates salt-sensitive hypertension in Dahl/ss rats. In conclusion, PHD2 regulation of HIF-1 $\alpha$ -mediated gene activation in the renal medulla is an important molecular adaptation to high salt intake; impaired PHD2 regulation of HIF-1 $\alpha$ -mediated gene activation in the renal medulla may be responsible for the salt-sensitive hypertension in Dahl/ss rats; correction of these defects may be used to as therapeutic strategies for the treatment of salt-sensitive hypertension.

**Keywords:** Salt sensitive hypertension, gene transfection, Dahl S rat, pressure natriuresis, hypoxia inducible factor-1 $\alpha$ , transcription factor, sodium excretion, heme oxygenase-1, cyclooxygenase-2, fluid homeostasis

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 known that renal medulla plays critical roles in the regulation of sodium excretion and long-term control of arterial blood pressure [5-7]. Many enzymes that produce natriuretic factors such as nitric oxide synthase (NOS), hemeoxygenase-1 (HO-1) and cyclooxygenase-2 (COX-2) are highly expressed in the renal medulla [8-14]. These enzymes in

the renal medulla are up-regulated in response to high salt intake [9, 12-15]. Inhibition of these enzymes within the renal medulla reduces sodium excretion and increases salt sensitivity of arterial blood pressure [9-12, 14, 16-18]. Moreover, in salt sensitive hypertensive animal models, the levels of these enzymes in the renal medulla are much lower [19-21] and the up-regulation of these enzymes in response to high salt diet and to angiotensin II are diminished [10, 19, 22-23] compared with normal controls. Thus, the protective factors produced by these enzymes in the renal medulla play critical roles in the regulation of sodium excretion via actions

on blood flow and tubular activity, which is essential in maintaining the homeostasis of body fluid volume and blood pressure. However, it remains a question what mechanisms mediate the activation of these enzymes in the renal medulla in response to high salt challenge.

### **HIF-1 $\alpha$ -mediated gene activation is an important molecular adaptation in the renal medulla in response to high salt challenge**

Recent studies have demonstrated that the genes encoding the protective factor-producing enzymes described above are oxygen sensitive genes and regulated by hypoxia-inducible factor (HIF)-1 $\alpha$  [24-26], a transcription factor whose level is also very high in the renal medulla [27-29] due to the low oxygen levels in this kidney region [30-33]. HIF-1 $\alpha$  is a master regulator of adaptation to hypoxia and activates gene transcription of many oxygen sensitive genes including NOS, COX-2 and HO-1 [24, 25, 34-36]. Activation of these genes in the renal medulla induces vasodilation and inhibits tubular activity, which promotes sodium excretion and consequently contributes to the control of blood pressure [6-8, 26, 37]. Therefore, renal medullary functions are in fact associated with genes that are transcriptionally regulated by HIF-1 $\alpha$ .

Although it was evidenced that the transcriptional expressions of the above enzymes were regulated by HIF-1 $\alpha$ , it remained unknown whether this HIF-1 $\alpha$ -mediated gene activation was of physiological relevance in the control of renal function, in particular the renal medullary function. We therefore performed a study to test the hypothesis that HIF-1 $\alpha$  mediates the activation of the oxygen sensitive genes such as NOS, COX-2 and HO-1 in the renal medulla, and thereby participates in the control of renal medullary functions and consequently regulates blood pressure [38]. In this study, we transfected HIF-1 $\alpha$  decoy oligodeoxynucleotides into the renal medulla to inhibit the binding activity of HIF-1 $\alpha$  and examined its effect on pressure natriuresis, renal cortical and medullary blood flows in response to the elevations of renal perfusion pressure (RPP) and sodium loading, and then determined its chronic effect on arterial blood pressure. It was demonstrated that blocking the transcriptional activity of HIF-1 $\alpha$  to inhibit the expression of its target genes in the renal medulla substantially blunted the increases of renal medullary blood flows and urinary sodium excretion in response to the eleva-

tions of RPP, suggesting that HIF-1 $\alpha$ , possibly through the actions on its target genes, is importantly involved in the regulation of renal medullary function. Since products of the enzymes encoded by these HIF-1 $\alpha$  target genes have been shown to dilate the medullary vasculature and inhibit the tubular activities [7, 37, 39, 40], the effect of HIF-1 $\alpha$ -mediated pathway on pressure natriuresis may be through both vascular and tubular actions.

To further evaluate the role of renal medullary HIF-1 $\alpha$  on salt handling, we examined the sodium excretion after acute sodium loading and the salt balance after chronic sodium challenge. The results from these experiments demonstrated that inhibition of HIF-1 $\alpha$ -mediated gene activation remarkably impaired the capability of the kidneys to remove extra sodium load, which resulted in sodium retention [38]. These data additionally suggest that renal medullary HIF-1 $\alpha$  is a regulator in sodium excretion. Since pressure-natriuresis and normal renal medullary function are key determinants to the long-term control of arterial blood pressure [6, 7, 37, 39, 41], our data further showed that inhibition of HIF-1 $\alpha$ -mediated gene activation led to a considerable increase in arterial blood pressure in response to high salt intake. Interestingly, inhibition of HIF-1 $\alpha$ -mediated gene activation did not produce hypertension when rats were not challenged with high salt. Therefore, high salt-induced activation of HIF-1 $\alpha$ -regulated pathways is considered as an adaptive mechanism to high salt intake, which results in an induction of various protective factors and consequent promotion of extra sodium excretion. Deficiency of HIF-1 $\alpha$ -mediated gene transcription in the renal medulla may decrease the production of various protecting factors, impair renal medullary function, prevent excretion of extra salt intake, consequently disrupt salt adaptation and increase the salt sensitivity of arterial blood pressure. These data suggest that HIF-1 $\alpha$ -mediated gene activation may be a common mechanism regulating the expression of various protecting factors in the renal medulla, thereby exerting an antihypertensive action when animals are exposed to high salt challenge.

### **HIF prolyl-hydroxylase-2 regulates HIF-1 $\alpha$ -mediated gene activation in the renal medulla in response to high salt challenge**

Our above studies suggest that HIF-1 $\alpha$ -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 and blood pressure control. However, it remains unclear how high salt intake induces the increases in HIF-1 $\alpha$  levels in the renal medulla. It has been recently demonstrated that HIF prolyl-hydroxylases are the major enzymes to promote the degradation of HIF-1 $\alpha$  [42-44]. HIF prolyl-hydroxylases catalyze site-specific proline hydroxylation of HIF-1 $\alpha$  using oxygen as a cofactor. After being prolyl-hydroxylated, HIF-1 $\alpha$  is recognized and targeted for degradation by the ubiquitin-proteasome pathway. Although HIF prolyl-hydroxylases work as oxygen sensor to regulate the destruction of HIF-1 $\alpha$  [42-44], recent evidence has clearly shown that the activities and expressions of HIF prolyl-hydroxylases are also regulated independent of oxygen levels by a variety of factors [45-49].

Three isoforms of HIF prolyl-hydroxylase, including prolyl hydroxylase domain-containing proteins 1, 2, and 3 (PHD1, 2, and 3), have been identified [42, 43, 50]. Previous studies including ours have demonstrated that PHDs are present in the kidneys with PHD2 as the predominant isoform of PHDs [51-55] and that PHD2 is most abundantly expressed in the renal medulla [51, 55]. Our data showed that i.p. injection of L-mimosine, a PHD inhibitor, for 2 weeks substantially upregulated HIF-1 $\alpha$  expression in the kidneys, especially in the renal medulla, and remarkably enhanced the natriuretic response to renal perfusion pressure in Sprague-Dawley rats [51]. Inhibition of HIF transcriptional activity by renal medullary transfection of HIF-1 $\alpha$  decoy oligodeoxynucleotides attenuated L-mimosine-induced enhancement of pressure natriuresis, which confirmed that HIF-1 $\alpha$  mediated the effect of PHD inhibitor. These results indicate that highly expressed PHDs in the renal medulla importantly contribute to the control of renal Na<sup>+</sup> excretion through regulation of HIF-1 $\alpha$  and its targeted genes [51].

Given the important role of PHDs in the regulation of HIF-1 $\alpha$  levels and renal function, we hypothesized that PHD2 responds to high salt intake and mediates high salt-induced increase of HIF-1 $\alpha$  in the renal medulla. We examined the effect of high salt intake on the expression of PHD2 and determined the role of PHD2 in high salt-induced activation of HIF-1 $\alpha$  by transfection

of PHD2 expression plasmids into the renal medulla. Our results showed that high salt intake decreased PHD2 levels in the renal medulla, and that over-expression of PHD2 transgene to disrupt high salt-induced PHD2 inhibition in the renal medulla blocked high salt-induced activation in HIF-1 $\alpha$  and its target genes, suggesting that high salt increases HIF-1 $\alpha$  level and thereby enhances expression of its target genes through inhibition of PHD2 [56]. Most recently, we further demonstrated that over-expression of PHD2 transgene to disrupt the high salt-induced inhibition of PHD2 and subsequently to inhibit the adaptive activation of renal medullary HIF-1 $\alpha$  in response to high salt challenge impaired renal medullary function and kidney salt handling, thereafter causing sodium retention and producing a salt sensitive hypertension [57]. These results demonstrated that high salt-induced activation of HIF-1 $\alpha$  and its target genes were associated with PHD2 inhibition and that over-expression of PHD2 transgene blocked the activation of HIF-1 $\alpha$  and its target genes after high salt challenge, suggesting that PHD2 functions as an upstream signal that regulates HIF-1 $\alpha$ -mediated gene activations in the renal medulla in response to high salt. It is concluded that PHD2 regulation of HIF-1 $\alpha$  in response to high salt in the renal medulla may represent a novel mechanism involved in renal salt handling and blood pressure regulation.

### **Impaired PHD2 regulation of HIF-1 $\alpha$ -mediated gene activation in the renal medulla in Dahl salt sensitive hypertensive rats**

The above information suggests that PHD2/HIF-1 $\alpha$  pathway is an important molecular mediator in renal salt adaptation under normal conditions. We wondered whether this PHD2/HIF-1 $\alpha$  pathway was involved in the pathogenic mechanism of abnormal renal sodium management in salt-sensitive hypertensive individuals. Dahl salt sensitive hypertensive (Dahl/ss) rat is a widely used genetic model of human salt-sensitive hypertension that exhibits many phenotypic characteristics in common with human hypertension [3, 58-61]. Renal medullary dysfunction is one of the major mechanisms for this rat strain to develop hypertension [6, 7, 10, 19]. Most interestingly, the above protective genes regulated by HIF-1 $\alpha$  has been shown to be impaired this animal model and deficiencies of these HIF-1 $\alpha$  target genes in the renal medulla are considered to be responsible for the development of

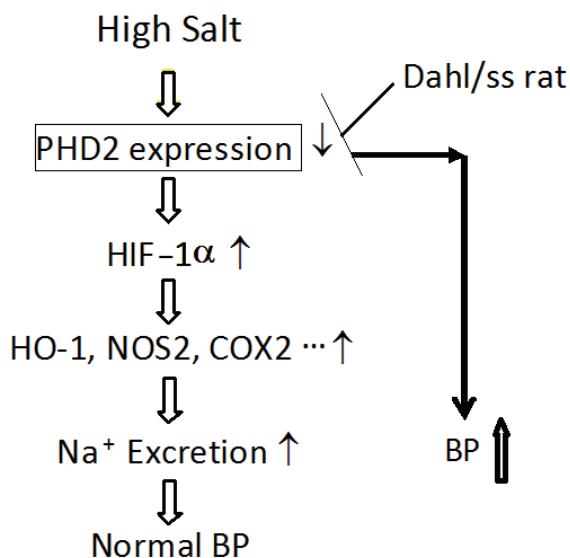
hypertension in Dahl/ss rats [10, 19-23]. For example, it has been reported that there is a defect in renal medullary NOS2, one of HIF-1 $\alpha$  target genes [19-21] in Dahl/ss rats, and that the activations of NOS2 in the renal medulla by a high salt diet and by angiotensin II are detected in normal animals but diminished in Dahl/ss rats [10, 19, 22, 23]. These studies indicate that there is possibly an impairment in renal medullary HIF-1 $\alpha$ , which produces the deficiencies in the expressions of NOS2 and other genes in the renal medulla in this rat strain.

We therefore determined whether the renal medullary HIF-1 $\alpha$  and PHD2 levels were altered in the renal medulla in response to a high salt diet in Dahl/ss rats. Our results showed a decreased expression and reduced response to high salt intake in HIF-1 $\alpha$  levels in the renal medulla from Dahl/ss rats compared with that from normal rats, which was accompanied by similar defects in HIF-1 $\alpha$  target genes HO-1, NOS2 and COX2 in Dahl/ss rats compared with normal rats [56]. These results indicate that HIF-1 $\alpha$ -mediated gene activations in these renal medullary protective factors are impaired in this rat strain. In parallel to these results, a higher level of PHD2 and failed inhibition of PHD2 in response to high salt intake in the renal medulla from Dahl/ss rats were observed [56]. Moreover, reducing PHD2 levels by shRNA restored the up-regulatory response to high salt challenge in HIF-1 $\alpha$  and its target genes HO-1, NOS2 and COX2 in the renal medulla in Dahl/ss rats [56]. It is suggested that diminished HIF-1 $\alpha$  in Dahl/ss rats is caused by abnormal PHD2 response to a high salt diet. These data additionally support the view that inhibitory response of PHD2 facing high salt challenge activates HIF-1 $\alpha$ -mediated gene expressions, consequently maintaining a sodium balance. Our results suggest that deficient PHD2/HIF-1 $\alpha$ -mediated molecular adaptation in response to high salt intake in the renal medulla may be the pathogenic mechanism responsible for producing salt sensitive hypertension this animal model.

We further investigated whether correction of the defects in PHD2/HIF-1 $\alpha$ -mediated molecular adaptation in response to high salt intake in the renal medulla would improve the renal sodium excretion and attenuate the salt sensitive hypertension in Dahl/ss rats. We induced the expression of HIF-1 $\alpha$  levels in the renal medulla by

local over-expression of HIF-1 $\alpha$  transgene or infusion of CoCl<sub>2</sub>, a HIF-1 $\alpha$  inducer, into the renal medulla and then determined the improvement of renal sodium handling and salt-sensitive hypertension in this animal model. Our results demonstrated that induction of HIF-1 $\alpha$ -mediated gene activation in the renal medulla increased the expression of anti-hypertensive genes in the renal medulla, and consequently enhanced the urinary sodium excretion, reduced sodium retention, as a result, attenuated the salt-sensitive hypertension in Dahl/ss rats [62]. It has been shown that high salt-induced activation of HIF-1 $\alpha$ -regulated 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 [38]. Therefore, deficiency of HIF-1 $\alpha$ -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/ss rats. This deficiency in HIF-1 $\alpha$ -mediated gene activation may represent an important mechanism for the development of salt sensitive hypertension. Induction of HIF-1 $\alpha$  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, which may be used to as a therapeutic strategy for salt-sensitive hypertension.

Because high salt-induced PHD2 inhibition and consequent HIF-1 $\alpha$  upregulation in the renal medulla was absent in Dahl/ss rats, we also tested whether silencing of PHD2 gene would increase the levels of HIF-1 $\alpha$  and its target genes in the renal medulla, consequently enhancing the sodium excretion and attenuating salt-sensitive hypertension in Dahl/ss rats. We transfected PHD2-shRNA plasmids into the renal medulla and then detected the renal sodium excretion and arterial blood pressure after high salt challenge in Dahl/ss rats. It was found that silencing of PHD2 gene to increase HIF-1 $\alpha$  levels in the renal medulla in Dahl/ss rats promoted sodium excretion and reduced sodium retention after sodium loading, and consequently attenuated salt sensitive hypertension [63]. It is suggested that the absence of inhibition in PHD2 in the renal medulla after high salt



**Figure 1.** HIF-1 $\alpha$ -mediated gene activation in the molecular adaptation to high salt intake in the renal medulla and its role in salt sensitive hypertension. High salt intake inhibits PHD2, an enzyme promoting HIF-1 $\alpha$  degradation, leading to the accumulation of HIF-1 $\alpha$  in the renal medulla. HIF-1 $\alpha$  then activates its target genes such as NOS2, HO-1 and COX-2, which augment the production of anti-hypertensive factors in the renal medulla to remove the extra sodium load, thereby, maintaining a normal blood pressure after high salt challenge. However, in Dahl/ss rats, high salt-induced inhibition of PHD2 is absent. Therefore, there is no HIF-1 $\alpha$  accumulation and neither the activation of anti-hypertensive genes in the renal medulla after high salt challenge, which impairs the capability of the kidneys to remove extra sodium load and results in sodium retention, consequently, producing a salt sensitive hypertension in this animal model.

challenge may represent a novel mechanism for the deficient HIF-1 $\alpha$  response and the development of hypertension in Dahl/ss rats, and that inhibition of PHD2 in the renal medulla could be used as a therapeutic approach for salt-sensitive hypertension.

**Summary**

Our serial studies have demonstrated that: 1) High salt intake stimulates HIF-1 $\alpha$ -mediated gene activation in the renal medulla. Blockade of this HIF-1 $\alpha$ -mediated gene activation in response to high salt challenge reduces sodium excretion, induces sodium retention and produces a salt-sensitive hypertension. It is suggested that HIF-1 $\alpha$ -mediated gene activation may increase the production of different renal

antihypertensive factors in the renal medulla, mediate renal adaptation to high salt intake and regulate salt sensitivity of arterial blood pressure. 2) PHD2, an enzyme that promotes the degradation of HIF-1 $\alpha$ , is the most abundant isoform of PHDs in the kidneys and highly expressed in renal medulla. High salt intake suppresses the expression of PHD2 in the renal medulla (Figure 1). This high salt-induced inhibition of PHD2 is an upstream signal that increases HIF-1 $\alpha$ -mediated gene expression in the renal medulla in response to high salt challenge. Disruption of this PHD2-associated adaptive activation of HIF-1 $\alpha$ -mediated gene expressions in the renal medulla blunts sodium excretion, induces sodium retention and increases salt sensitivity of blood pressure. Thus, PHD2 regulation of HIF-1 $\alpha$ -mediated gene activation in the renal medulla is one of the important adaptive mechanisms to high salt challenge and thereby participates in the control of salt sensitivity of blood pressure. 3) The high salt-induced inhibition in PHD2 and the consequent activation of HIF-1 $\alpha$  in the renal medulla is absent in Dahl/ss rats. Induction of HIF-1 $\alpha$  levels in the renal medulla in Dahl S rats increases the expression of anti-hypertensive genes, enhances sodium excretion, reduces sodium retention and attenuates the salt-sensitive hypertension. Meanwhile, inhibition of PHD2 in the renal medulla in Dahl S rats corrects the defect in HIF-1 $\alpha$ -mediated molecular adaptation, improves sodium excretion, reduces sodium retention and attenuates salt-sensitive hypertension. In conclusion, PHD2 regulation of HIF-1 $\alpha$ -mediated gene activation in the renal medulla is an important molecular adaptation to high salt intake; impaired PHD2 regulation of HIF-1 $\alpha$ -mediated gene activation in the renal medulla may be responsible for the salt-sensitive hypertension in Dahl S rats; correction of these defects by inhibiting PHD2 or inducing HIF-1 $\alpha$  may be used as therapeutic strategies for the treatment of salt-sensitive hypertension. It remains unanswered how high salt inhibits PHD2 expression in the renal medulla. Our interesting finding that the expression of the PHD2 transgene is decreased by high salt intake [56] indicates that high salt-induced PHD2 inhibition may be associated with a reduced mRNA stability. The exact mechanisms for high salt to inhibit PHD2 require further exploration.

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**Address correspondence to:** Dr. Ningjun Li, Department of Pharmacology & Toxicology, Medical College of Virginia Campus, Virginia Commonwealth University, P.O. Box 980613, Richmond, VA 23298 Tel: (804) 828-2071; Fax: (804) 828-4794; E-mail: nli@vcu.edu

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