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NO and the A and B of Endothelin of Sodium Homeostasis

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Abstract

Purpose of review—In recent years, renal collecting duct specific endothelin (ET1), ETA and ETB receptors as well as nitric oxide synthase 1 (NOS1) knockout mice have been developed with subsequent identification for an integral role in regulation sodium water homeostasis and ultimately blood pressure. The focus of this review is to integrate these models and to propose a scheme for the control of sodium excretion by the collecting duct and the ET/ETB/NOS system.

Recent findings—NOS1 splice variants are expressed in the kidney, especially the collecting duct (CD). Mice express predominantly NOS1 β in the medulla with NOS1 α and NOS1 β in the cortex while rats express NOS1 α and NOS1 β in both the cortex and medulla. ET1 via the ETB receptor increases NO production in both rat and mouse collecting ducts, suggesting that NOS1 β is linked to ET1 dependent NOS activation in the kidney. As well, genetic deletion of NOS1 splice variants in the collecting duct results in a salt-sensitive hypertensive phenotype in mice, much like the CD ET1 and CD ETB knockout mice.

Summary—In the CD, the ET1/NO pathways are intimately linked, and deletion of CD ET1, ETB receptor, or NOS1 β , results in a salt sensitive phenotype, which is at least partially dependent on dysregulation of sodium and water reabsorption.

Keywords

endothelin; Nitric oxide; Nitric oxide synthase; splice variants; sodium and water excretion

Introduction

In recent years, advances have been made in the research area of sodium and water homeostasis through the development of collecting duct (CD) principal cell knockouts. These include genetic deletion from the CD endothelin-1 (ET1), endothelin A receptor (ETA) and endothelin B receptor (ETB). Most recently, we deleted nitric oxide synthase-1 (NOS1) from the CD of the mouse. From these different models, it is evident that ET1/NO signaling in the CD regulates sodium and water homeostasis. Clinically, what these studies have directly illuminated is that CD ET1/NO is necessary for blood pressure regulation.

NO and NOS in the collecting duct

In the mouse CD, NOS1 (neuronal NOS; nNOS) and NOS3 (endothelial NOS; eNOS) are expressed. Additionally, NOS2 (inducible NOS) mRNA was described from the rat inner medullary CD (IMCD) [1]. The IMCD has the greatest total NOS activity out of all the regions of the rat kidney [1]. Physiological stimuli, such as an increase in shear stress, lead

to significant increases in IMCD NO production [2]. Shear stress in the tubule is increased in states of increased urine flow, such as are experienced during consumption of high salt diets [2]. Interestingly, infusion of NOS1 anti-sense oligonucleotides or NOS1 pharmacological inhibitors into the medulla of the rat, results in a significant increase in blood pressure when the rats were fed a high salt diet [3]. Surprisingly, the commercially available total body NOS1 (exon 2) knockout mouse is normotensive, even on a high salt diet [4]. What is still not well understood in the field, is that NOS1 expresses multiple splice variants (Fig 1). NOS1 α is the full length variant and the first described NOS enzyme with a molecular weight of 160 kDa. NOS1 β and NOS1 γ are alternatively spliced and lack exon 2 thus producing N-truncated variants that lack a PDZ domain, and have a molecular weight of 130 kDa and 125 kDa, respectively [5,6]. These splice variants are differentiated by molecular weight with western blots and the use of C-terminus antibodies. NOS1 β possesses activity comparable to NOS1 α , while NOS1 γ has only ~3% of the enzymatic activity [5,6]. Additionally, there is a NOS1 splice variant with a 102 bp insertion between exon 16 and 17, resulting in a 34 amino acid addition [7]. This variant is termed NOS1 μ and is expressed in skeletal muscle and heart [7]. NOS1 splice variants have been described in the kidney, with a high proportion of the mRNA being NOS1 β [8]. Recently, it was determined that the rat cortex [9] and macula densa [10] express NOS1 α and NOS1 β . Furthermore the rat medulla [9] and inner medullary CD (IMCD) [11] express NOS1 α and NOS1 β . However, in the mouse IMCD or in mIMCD-3 cells (an *in vitro* mouse IMCD cell culture line), predominantly NOS1 β is expressed [11]. The commercially available NOS1 knockout mouse is a NOS1 α knockout mouse because it was targeted at exon 2 [12]. A total NOS1 knockout mouse (targeted at exon 6) was developed but these mice have severe developmental abnormalities and only survive on a liquid diet [13] however there are no reports of blood pressure in these mice.

During high dietary salt intake, urinary NO_x (nitrite + nitrate) excretion, an index of NO production, is increased in mice [14–18], rats [19–21] and in humans [22–24]. Low levels of urinary NO_x excretion correlates with the progression of hypertension in humans [25]. Recent studies have aimed to determine if the NOS1 splice variants are regulated by dietary sodium. During challenge with a high salt diet, NOS1 β expression is increased in the rat macula densa, and NOS1 α is reduced [10], suggesting that NOS1 β is a salt sensitive splice variant. Additionally, Lu et al. [10], found a significant increase in macula densa NO, putatively derived from NOS1 β , and attenuation of tubuloglomerular feedback mechanism. This suggests that high salt diet induced NOS1 β -derived NO regulates sodium delivery through the nephron and sodium excretion, although this hypothesis needs to be tested directly. We have developed a CD NOS1 (exon 6) knockout mouse, and these mice display a salt-sensitive blood pressure phenotype (submitted paper: Hyndman *et al.* unpublished data). Moreover, switching the diet from sodium deficient to a high salt diet in the CDNOS1KO mice, results in a significant blunting of urinary sodium excretion, NO_x excretion, and urine production (submitted paper: Hyndman *et al.* unpublished data). Thus we speculate that like the macula densa, CD NOS1 β is critical in sodium and water homeostasis. We have further found that expression of the epithelial sodium channel (ENaC) is significantly higher in the membrane fraction of renal tissue homogenates of the CDNOS1KO mice on a high salt diet compared to flox control mice (submitted paper: Hyndman *et al.* unpublished data). These data suggest that NOS1 in the collecting duct may directly regulate the trafficking of sodium channels and/or other transporters.

Endothelin-1 in the kidney

Endothelin-1 (ET1) and the endothelin receptors, are most highly expressed in the renal collecting duct, where ET1 functions as diuretic/natriuretic factor. Recent studies have determined that CD ET1 production is regulated by flow, Na delivery, the epithelial sodium

channel, and mitochondrial Na⁺/Ca⁺ exchanger [26,27**]. Thus, extracellular fluid expansion and resultant increase in renal tubular flow (likely shear stress) are important physiological regulators of CD ET1 production. Interestingly, the role of mitochondrial Na⁺/Ca⁺ exchanger in the regulation of ET1 production is novel, and will likely be the focus of many studies to come.

Recent work by the Kohan lab, has developed four principal cell CD knockout mice of the endothelin system. They have genetically deleted ET1 [28,29], ETA receptor [30], ETB receptor [31] and both ETA and ETB (ETA/B) receptors [32]. These studies were recently reviewed [33] but to summarize deletion of CD ET1, ETB or ETA/B results in sodium retention and a significant salt-sensitive increase in blood pressure. Deletion of the ETA receptor had little effect on sodium excretion or blood pressure [30]. CD ET1 KO mice have an impaired ability to excrete a sodium load [29]. Furthermore, the CD ETB KO mouse, displays a blunted sodium excretion when given an acute salt load (i.p.) in the first 4 h after injection compared to flox control animals [31]. CD ETB KO mice also present with reduced plasma renin activity on both normal and high salt diets, suggesting that there is volume expansion in this model. Thus, CD ET1 and ETB are integral in sodium balance and blood pressure regulation.

To examine the mechanism of the increased sodium retention in these models, a series of cortical CD (CCD) patch clamp studies were completed to determine the effect of the ET1 system on ENaC activity. Exogenous ET1 significantly reduces ENaC activity (as measured by open probability, P_o) in CCD of wild type mice [34*] and rats [35]. The inhibition of ENaC by ET1 involves the src-ERK1/2 pathway [35]. Moreover, the CCD of the CD ETB KO mice and CD ETA/B KO mice have increased P_o compared to CCD from wild type and CD ETA KO mice [34*]. Thus, ET1 via the ETB receptor in the CD inhibits ENaC activity and is, at least partially, part of the mechanism of sodium retention and salt-sensitive hypertension in the CD ET1 KO models.

Endothelin-NO interactions in the CD

The endothelin-NO systems interact in the renal collecting duct. In mIMCD-3 cells, ET1 via the ETB receptor increases NO production but did not change NOS1 or NOS3 expression [36]. ET1 via ETB receptors also increased phosphorylation of ERK1/2, however this pathway is independent of ETB-mediated NO production, as inhibition of ERK1/2 phosphorylation does not prevent the ET1 induced increase in mIMCD3 NO production [36]. Currently, it remains to be determined what the mechanism of intracellular signaling that links ET1/ETB to NOS1 and NOS3 in the IMCD. It is possible that this is regulated by intracellular calcium, as has been shown in the rat thick ascending limb [37]. Moreover, it could be regulated by protein-protein interactions, as we recently reported that dynamin-2 interacts with CD NOS1 α and NOS1 β in the rat collecting duct, and NOS1 β in the mIMCD3 leading to a significant increase in NO production [11]. Preliminary experiments suggest that this interaction is regulated by intracellular calcium, whereby an increase in intracellular calcium significantly increases the dynamin-2/NOS1 interaction and NO production [Hyndman and Pollock, unpublished]. We are currently testing the hypothesis that ET1 regulates the dynamin-2/NOS1 interaction in the IMCD and that this cascade is dependent on intracellular calcium (Fig. 2). Finally, the role of dynamin/NOS interactions in sodium homeostasis needs to be addressed, and may be a novel intervention for increasing endogenous NO in models deficient in NO, such as chronic kidney disease [9].

In the rat inner medullary CD (IMCD) suspensions, ET1 via the ETB receptor increased NOS1-derived NO production [38]. In these studies, it was not determined if this increase in NO was from NOS1 α or NOS1 β , as both splice variants are expressed in the rat CD [11]. In

the CD ET1 KO mice, there is a significant reduction in urinary NO_x excretion (NO metabolites) on both normal and high salt diets [39], again illustrating that ET1 basally signals through NOS1 β in the mouse CD (NOS1 β is the major splice variant in the mouse CD [11]). CD ET1 KO mice on a high salt diet have reduced inner medullary NOS1 and NOS3 activity, but no differences in protein expression [39]. CD ET1/NO signaling pathway modulates pressure-natriuresis, as the CD ET1 KO mouse displays a significant blunting in sodium and NO_x excretion when subjected to acute increases in blood pressure (provided by tightening ligatures around the celiac and mesenteric arteries) [39]. Our current studies are examining whether CD NOS1 KO mice have a shifted pressure-natriuresis relationship to further support the hypothesis the CD NOS1-derived NO is a modulator of the pressure-natriuresis relationship.

A scheme of the ET-1/NO interactions leading to regulation of sodium excretion in the collecting duct is depicted in figure 2.

Conclusion

With the development of CD-specific ET and NOS1 knockout models, the roles of ET1 and NO in the regulation of sodium homeostasis have been illuminated. These models allow hypotheses to be tested on the cellular mechanisms of CD sodium transport, and ultimately may lead to novel pathways for the treatment of salt-sensitive hypertension. Moreover, determining the physiological role of the NOS1 splice variants and their contribution to renal function in all parts of the kidney, is an important next step to further our knowledge of NO diuretic and natriuretic effects. Many questions of how the NOS1 splice variants are regulated and their interaction with the endothelin system in all areas of the kidney need to be elucidated to fully “kNOw” the “A and B of endothelin” signaling of sodium homeostasis and blood pressure control.

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Figure 1.
Predicted NOS1 splice variant mRNA from the mouse and predicted protein domains
(modified from [6])

Figure 2.

A schematic of the interactions of the endothelin-1 (ET1) and nitric oxide (NO) systems in the regulation of sodium excretion in the collecting duct during an increase in sodium delivery through. Dash lines represent hypothetical links. i – intracellular.