Salt control. Focus on “High salt induces autocrine actions of ET-1 on inner medullary collecting duct NO production via upregulated ETB receptor expression”

© Armin Just
Physiologisches Institut, Albert-Ludwigs-Universität, Freiburg, Germany
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SALT AND WATER HOMEOSTATIS is an essential everyday business for any animal. The inner workings, however, of how accurate salt and water balance is achieved in an environment of ever-changing availability of salt and water, is highly complex and not entirely understood (4). In the current issue of the American Journal of Physiology—Regulatory, Integrative and Comparative Physiology, Hyndman et al. (14) provide essential building blocks to assemble an important part to this vast overall puzzle. The authors elucidate the role of endothelin-1 (ET-1), ETA and ETB receptors, nitric oxide (NO), and sodium reabsorption via the epithelial Na+ channel (ENaC) in the inner medullary collecting duct (IMCD) of the kidney under the challenge of a high-salt intake by providing essential new information regarding this regulatory system.

Volume homeostasis is basically a function of the amount of sodium in the extracellular fluid. This is the consequence of the requirement to maintain osmotic pressure within narrow limits to avoid major fluid shifts between the intracellular and extracellular compartments, combined with the fact that this osmoregulation is achieved by adapting the amount of water secondary to that of solutes. The recent evidence that sodium may be accumulated in the skin at higher concentrations than in the remainder of the extracellular space (33) adds some additional complexity. However, although this provides an elegant way for buffering dynamic changes in salt intake without the need for equally sized changes in extracellular fluid volume, such cutaneous salt stores unlikely have unlimited capacity and will, thus, ultimately not alter the basic concept of regulation of volume via the amount of sodium. Accordingly, a multitude of regulatory systems exist—both locally within the kidney and systemically within the entire organism—regulating the ingestion and renal excretion of sodium—both with and without involvement of pressure natriuresis, and thus with and without salt-sensitive hypertension (4). One of these sodium regulatory systems is provided by ET-1 in the inner medulla of the kidney (16–19).

Endothelins act via two G protein-coupled receptors, ETA and ETB receptors (19, 25). ETA receptors preferentially bind ET-1, while ETB receptors have similar affinities for ET-1, ET-2, and ET-3 (7, 19). Like endothelin itself, ETA and ETB receptors are most prominently expressed in the renal inner medulla (16–19).

NO is produced by NO synthase isoforms NOS-1 (neuronal), NOS-2 (inducible), and NOS-3 (endothelial) (2). All three NOS isoforms are expressed in the kidney (20, 31). In general, NOS-1 is found in the macula densa and the collecting duct, NOS-3 in the endothelium of renal arteries and arterioles, including afferent and efferent arterioles and vasa recta and also in tubular cells of the proximal tubule, thick ascending limb, and collecting duct, and NOS-2 also seems to be expressed in the proximal tubule and IMCD, even in the intact kidney without inflammation, although NOS-2 protein detection has remained controversial in the intact kidney (20, 31). For NOS-1, four splice variants exist (2). Variant NOS-1γ has negligible enzymatic activity, and NOS-1α is confined to heart and skeletal muscle (2). Variants NOS-1α and NOS-1β are both found in the kidney, with NOS-1β-expression being upregulated during pregnancy (28), high-salt feeding (22), aging (24), and in a model of chronic renal failure (27). The primary receptor for NO is soluble guanylate cyclase producing cGMP in the target cells (9). Interestingly, cGMP production in response to ACh or NO-donors in the kidney has been found most prominently in the renal medulla (21).

The role of endothelin in the renal inner medulla for sodium homeostasis has been investigated in detail over the years, as laid out in several recent review articles (8, 16–19, 30). Collecting duct-specific gene knockout of ET-1 (CDET1-KO) (1, 26), ETB (CDET1B-KO) (10), ETA receptors (CDET1A-KO) (12), or both ETA + ETB receptors (CDET1A-B-KO) (11), and of NOS1 (CDNOS1-KO) (13) have been instrumental in dissecting out the model. The diuresis induced by a challenge with free water and the natriuresis after a load of sodium are both associated with enhanced excretion rate of ET-1 in the urine (reviewed in Ref. 18), although in a study on humans, small acute sodium loads failed to affect urinary ET-1 excretion (3). Similarly, sodium loading leads to enhanced urinary excretion of NO-metabolites (reviewed in Ref. 16), CDET1-KO (1, 26), CDET1B-KO (10), and CDET1A-B-KO (11) all show hypertension under baseline conditions, salt-sensitive exaggeration under chronic high-salt feeding, and an impaired ability to eliminate an acute sodium load. In contrast, CDET1A-KO mice are neither hypertensive, nor salt-sensitive (12). However, additional deletion of ETB receptors in CDET1B-KO mice, leads to stronger hypertension at normal and high-salt conditions than the deletion of ETB receptors alone (CDET1B-KO), indicating a
contribution of ET$_A$ receptors and interactions between the receptor subtypes (4) (reviewed in Refs. 8 and 17). Other studies have shown that ET-1 stimulates NO and cGMP production in IMCD cells, predominantly via NOS-1 (32). The functional importance of collecting duct NOS-1 is revealed in CDNOS1-KO mice that are normotensive under standard diet, but develop salt-sensitive hypertension in response to elevated salt intake (13). It is noteworthy for the overall picture, however, that salt-sensitive hypertension is also induced by cell-selective NOS-1 deletion confined to a tiny, completely different nephron segment, i.e. the macula densa (23). Sodium reabsorption in the IMCD is mediated primarily via the epithelial sodium channel (ENaC) (29, 34), and ET-1 inhibits the open probability of ENaC (2, 16, 18, 30). In the collecting duct, this effect is mediated primarily via ET$_B$ receptors (5) and involves signal transduction via src kinase and MAPK1/2 (6).

NO is also involved in the IMCD response to high salt, as demonstrated by the mentioned salt sensitivity of CDNOS1-KO animals (13). ET-1 is known to stimulate NO release in IMCD via ET$_B$ receptors (16–18). However, the role of NO in the signal transduction between ET$_B$ receptors and ENaC is not entirely clear, because in contrast to the mentioned role of MAPK1/2 between ET$_B$ receptors and ENaC, ET$_B$-mediated NO release in IMCD is not affected by the same pathway (15), and it is uncertain whether NO can directly affect ENaC (29).

Although high-salt intake is known to enhance IMCD ET-1 production, another open question was whether salt intake might also modulate the IMCD expression of ETA and ET$_B$ receptors. Unclear was also whether the ET-1 responsible for IMCD NO stimulation and ENaC inhibition is solely derived from IMCD cells themselves or might also be influenced by ET-1 from the endothelium of nearby vasa recta.

The study by Hyndman et al. (14) in this issue addresses these important missing details in the regulatory system of the IMCD. In a broad methodological approach from conscious animals to inner medullary tissue, isolated IMCDs, and primary cultures of IMCD cells, the authors investigate tail-cuff blood pressure, ET receptor binding, mRNA-expression, NO production, and transepithelial resistances in wild-type mice, CDET1-KO, and mice with cell-selective knockout of ET-1 in vascular endothelial cells (VEET-KO). The major findings include the following: 1) High-salt diet shifts the relative amounts of cell surface ET$_A$ and ET$_B$ receptors within the inner medulla from a baseline distribution of 1:2 with normal food toward 1:10 ET$_B$-predominance with unaltered total receptor number and unaltered receptor mRNA expression during high-salt intake. 2) The inhibitory effect of ET-1 on ENaC permeability, as estimated from amiloride-sensitive transepithelial resistance of cultured IMCD, is mediated via ET$_B$ receptors and NOS-1 and maybe NOS-3, but not via ET$_A$ or NOS-2. 3) High-salt intake leads to enhanced NO (nitrate) production by isolated IMCD, which depends entirely upon ET-1 from the collecting duct, but not from the endothelium, because the effect was eliminated in CDET1-KO, but unaltered in VEET-KO. High-salt-induced NO production was also blocked by acute ET$_B$ antagonism, but it was not associated with significant changes in the expression levels of NOS-1$\beta$ or NOS-3 in IMCDs. Interestingly, the absence of ET-1 from all endothelial cells (not only from vasa recta) in VEET-KO did not affect tail-cuff systolic arterial pressure in either a normal or high-salt diet. Although these tail-cuff measurements would need later confirmation by telemetry, they do, by comparison, further underline the importance of ET-1 from the collecting duct for arterial pressure.

The major question remaining is how this piece of the puzzle belongs into the overall picture of sodium homeostasis. The foremost question herein is, how the IMCD would sense an excess of sodium in the body. Hyndman et al. (14) provide an interesting hypothesis, i.e. that ET-1-release from the IMCD is being stimulated by enhanced tubular flow, which then would lead to enhanced tubular shear stress and subsequently ET-1 release. However, for an increase in total body sodium to enhance volume flow in the IMCD without any other primary change in sodium reabsorption, the sodium load would need to be accompanied by an equal or at least similar increase in water intake. However, in the harsh reality of real life outside the laboratory animal facility, water may not always be available in sufficient amounts, which would then make sodium homeostasis rather unreliable. Of course, if water intake falls short, then fluid will osmotically come in from the intracellular space. However, since intracellular space is about twice as large as the extracellular, only two-thirds of the required water would actually enter and, hence, osmotic pressure would remain higher than before the sodium load. That scenario would probably stimulate vasopressin release despite the concomitant hypervolemia, which would then enhance water reabsorption and thus reduce, not enhance, IMCD volume flow. As reviewed in Refs. 17 and 18, the IMCD-ET-system does not seem to be affected by vasopressin, bradykinin, or norepinephrine. However, aldosterone and ANP would still seem attractive modulating candidates, since the IMCD is a known target for these hormones regarding sodium reabsorption (34). On the other hand, flow in the IMCD might, indeed, increase after a sodium load, if other regulatory systems would enhance sodium excretion in tubular segments preceding the IMCD and if vasopressin would match this by appropriate water excretion. In that case, the IMCD-ET-NO-ENaC-system might provide a final amplifier to the action of all such upstream mechanisms, including a feature for adjustable amplification power. If the amplifier gain of the IMCD would be too low or missing (e.g., as in CDET1-KO), the strength of the upstream regulatory systems alone might become so small, that only pressure diuresis would gain sufficient strength over time to achieve sodium balance, but at the expense of salt-sensitive hypertension. The adjustable gain would also allow to sensitize the IMCD amplifier and, thereby, the entire regulatory system, under conditions of chronic high-salt intake. The results of Hyndman et. al (14) would suggest, that such high-salt-induced amplification is due to enhanced ET-1 production by IMCD cells (induced by a mechanism that is upheld for at least the duration of the tissue preparation), and probably is also due to the observed shift toward ET$_B$ predominance in the ET receptor population on IMCDs.

It may be a long time until all of the details are clarified regarding the integrative function of the IMCD-ET-NO-ENaC-system within overall sodium homeostasis, but the meticulous analysis by Hyndman et al. (14) provides a major step advancing our understanding of this issue.
REFERENCES


