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Salt, salt-sensitivity and the endothelium: a pathway to discovery of molecular mechanisms

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This issue of *Hypertension* contains a report of molecular mechanisms transducing dietary salt intake which may have broad clinical relevance. These molecular mechanisms are complex, but to begin to understand this study we have to understand the importance of dietary salt intake and salt-sensitivity. This background will be discussed in some detail before discussing the molecular mechanisms.

Although cohort studies have called into question the role of dietary salt intake in causing hypertension (1), cause and effect relationships are most convincing when demonstrated using randomized clinical trials. It would be unethical to perform a randomized clinical trial in which the dietary intake of salt (sodium chloride) is increased over years in humans to observe an increase in blood pressure. However, such an experiment in primates has been performed by Denton et al who studied in Gabon, Africa, 26 chimpanzees (2). Chimpanzees have a native diet that is vegetarian and is very high in potassium content. The baseline sodium intake in these primates was only 6 mEq/d; in contrast that of K was 235 mEq/d. Blood pressure was measured in the 26 animals for one year. Subsequently, among half the animals, increasing amounts of salt (in increments of 5 g/d sodium) were added to a diet to a steady state level of 15 g/d. This dietary intake was maintained over 16 months. After this period of dietary supplementation, the diet was switched to baseline and observations made over another 6 months. A dose response relationship between salt supplementation and blood pressure increment was seen. With 15 g/d sodium supplementation over 67 weeks, the change from baseline in blood pressure was 33/10 mmHg. In comparison, 5 g/d sodium supplementation over 19 weeks provoked blood pressure change of 12/0 mmHg. Notably, plasma renin activity was reduced with salt supplementation, suggesting that volume expansion may have been a mechanism to induce hypertension. These experiments support the notion that dietary salt supplementation increases blood pressure in primates.

It has also been recognized that response to increasing dietary salt intake is not always accompanied by hypertension. Some people, despite increasing salt intake to very high levels, remain normotensive. This was recognized a long time ago by Louis Dahl who hypothesized that the blood pressure response to an increase in salt intake is an inherited trait (3). He reasoned that if such a trait existed, it would be possible to segregate these genes by inbreeding. However, if such a trait was environmental, inbreeding experiments

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would not be able to separate out animals who are predisposed and those who are protected for hypertension in response to a high salt intake. In this classic paper published in 1962, Dr. Dahl produced evidence to demonstrate that genetic factors play an important role in susceptibility to experimental hypertension (3). The blood pressure response to high salt diet in animals is normally distributed. Thus, there would be individuals who have extreme increase in blood pressure or little increase in blood pressure. Dr. Dahl reasoned that these extreme responses may be genetically determined. If response to blood pressure was inherited, then it should be possible to segregate the genes by inbreeding experiments. As a first step he fed Sprague Dawley rats a high salt diet and measured serial blood pressures. The blood pressure response was compared to animals given a low salt control diet to demonstrate the response of salt on blood pressure. He then discovered that thyroid injections and a high salt diet would uncover the blood pressure response within 4 days. Blood pressure in thyroid-salt treated animals was much higher compared to a control group. Also, those animals on the high salt diet stayed hypertensive at the end of 1 month and 1 year compared to controls. In the high salt fed animals, those that remained normotensive or hypertensive were selected and inbred for three generation. In the normotensive group, increasing resistance in response to a high salt intake could be demonstrated in blood pressure response over three generations. In the hypertensive group, increasing blood pressure susceptibility to high Na intake could be demonstrated with successive generations.

These experiments strongly support the role of genetic susceptibility to the development of experimental hypertension from excess salt ingestion. Dahl, in his original experiments also reported that pneumonia swept the rat colony, preferentially killing the salt-sensitive rats. Could there be more than volume to the salt-sensitivity story? What is the underlying pathophysiology of hypertension in these animals?

These questions were asked by the group of Dr. Paul Sanders at University of Alabama at Birmingham in the early 90's (4). Young Dahl rats were randomized in a 2 × 2 design to receive either a low salt or high salt diet. Blood pressure was measured at baseline and also after infusing a drug, N^G-monomethyl-L-arginine (L-NMMA), to block endothelial nitric oxide synthase. Elevation in blood pressure would then be a reflection of nitric oxide synthase activity. Thus, L-NMMA response to blood pressure was used as a biomarker of NO activity.

In the Dahl salt-resistant rats, baseline levels of MAP were similar despite high salt feeding for two weeks; an expected response, given that these animals are resistant to salt feeding. A 21% increase in MAP was seen in animals treated with low Na diet but a 31% increase with high Na diet. These data suggest that NO production was stimulated with high salt feeding in salt-resistant animals. This fact was uncovered by greater increase in MAP with L-NMMA in high Na treated animals.

Further experiments revealed that salt-sensitive animals appear to be different from salt resistant animals in that no NO stimulation occurs with Na feeding. This raises the possibility that these animals have a defect in endothelial function that underlies the salt sensitivity.

Awake unrestrained salt-sensitive rats did not develop hypertension when given L-arginine orally despite consuming a high salt diet. In contrast, D-arginine treated animals were significantly hypertensive by day 2. Prevention of hypertension occurred in a dose-response fashion. L-arginine did not lower blood pressure in the salt-resistant animals. In separate experiments, L-arginine supplementation did not prevent the development of hypertension in the spontaneously hypertensive rats indicating this mechanism to be specific to the Dahl rats. These observations were confirmed further by demonstrating that intraperitoneal injections of L-arginine in high salt fed salt-sensitive animals did not result in the development of hypertension. In contrast, injections of D-arginine did not protect from increased MAP. In L-arginine treated animals, L-NMMA infusion increased MAP 45% from baseline. In contrast, among D-arginine treated animals MAP increased only 18%. These data suggested that stimulation of NO production occurred with L-arginine feeding that restored the endothelial response to high salt feeding strongly pointing to endothelial dysfunction being causal in the pathogenesis of salt-sensitive hypertension.

Translation of the above findings to humans was provided by a study from Spain, in which 19 patients with salt resistant hypertension confirmed by ambulatory blood pressure monitoring were compared to 26 patients with salt sensitive hypertension (5). Although maximal vasodilatory responses to sodium nitroprusside were similar in the two groups, endothelium-dependent vasodilatation was less in the salt-sensitive group. L-NMMA produced a greater change in forearm blood flow in the salt-resistant group. The endothelium may also mediate the renal and systemic hemodynamic responses among healthy humans given a high salt diet (6). Specifically, compared to a low salt diet period, among 12 healthy volunteers participating in a cross-over trial, L-NMMA resulted in a greater change from baseline in renal and systemic hemodynamics in subjects given a high salt diet. Taken together, these data suggest that (i) the endothelium modulates the renal and systemic response to salt and (ii) that the endothelial function is impaired in people with salt-sensitive hypertension.

From the discussion so far it is clear that the response to salt intake in populations is variable. Some members of the population remain normotensive despite a high salt intake. The missing link appears to be the vascular response. Those who are unable to vasodilate have a hypertensive response. Thus, although the endothelium can “see” the salt, how does it do so?

Although the endothelium has many eyes, my discussion is focused to a few pathways. The Sanders lab has demonstrated that the production of the profibrogenic cytokine, TGF-β1 is increased in rodents fed a high salt diet within 2–4 days at a time point when volume expansion may not have occurred (7). This leads to an important and testable hypothesis whether high salt intake initiate damage to the vasculature independently of blood pressure. To dissociate the effects of pressure from direct damage molecular techniques in cultured cells have provided important insights.

In the current issue Hypertension, the group led by Paul Sanders examines such molecular mechanisms using multiple lines of investigations from using whole animal models to cells in culture. They first demonstrate that compared to rats fed a low salt diet (0.3%), within 4

days of feeding young Sprague Dawley rats with a high salt diet (8%) several endothelial effects were seen. These included an increase in several endothelial proteins such as TGF-β and phosphorylated Smad2 (Figure). Downstream, phosphorylation of Smad2 provoked phosphorylation of both Akt and the endothelial isoform of nitric oxide synthase (NOS3) but decreased the concentration of Phosphatase and tensin homologue deleted on chromosome 10 (PTEN). After feeding high salt diets for 2 days to these animals followed by another 2 days of a specific inhibitor of the TGF-β receptor 1/Activin receptor-like kinase 5, phosphorylation of Smad2 was blocked to levels seen in animals fed a low Na diet (0.3%). Furthermore, downstream events were also abrogated.

Next, to further understand the role of TGF-β1 on blood vessels, experiments in macrovascular endothelial cells were performed. Treatment of these cells with TGF-β1 increased phosphorylated NOS3 and as expected the concentration of nitric oxide metabolites in the medium. However, such effects were abolished by blocking PTEN via siRNA. Akt activation and NOS3 phosphorylation increased when PTEN was blocked; supplementing TGF-β1 in this setting provided no additional effects on Akt, NOS3, or nitric oxide production.

These experiments reveal the complex interaction of salt with endothelial cells on TGF β1 production, stimulation of PTEN, and neutralization of the TGF-β1 effect on the vasculature by increased production of nitric oxide. Observations made in this study may be of great clinical relevance. For example, several molecular lesions can be postulated that may provoke vascular injury in response to a high salt diet. These include a robust production in the vasculature of TGF-β1, lack of PTEN response, impaired Akt activation and NOS3 phosphorylation culminating in reduced nitric oxide production. These pathological responses that likely will provoke vascular injury may occur due to genetic polymorphisms or acquired defects in these pathways. The latter may be seen with chronic kidney disease, increasing age, or other conditions associated with salt sensitivity. On the other hand, these pathways may be amenable to pharmacological intervention and increase our ability to protect the vasculature among the elderly or those with chronic kidney disease. Furthermore, it illustrates the perseverance of a group of investigators who with continued federal support have increased our understanding of the molecular basis of salt sensitivity.

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Reference List

1. Stolarz-Skrzypek K, Kuznetsova T, Thijs L, Tikhonoff V, Seidlerova J, Richart T, Jin Y, Olszanecka A, Malyutina S, Casiglia E, Filipovsky J, Kawecka-Jaszcz K, Nikitin Y, Staessen JA. Fatal and nonfatal outcomes, incidence of hypertension, and blood pressure changes in relation to urinary sodium excretion. JAMA. 2011; 305:1777–1785. [PubMed: 21540421]
2. Denton D, Weisinger R, Mundy NI, Wickings EJ, Dixson A, Moisson P, Pingard AM, Shade R, Carey D, Ardaillou R. The effect of increased salt intake on blood pressure of chimpanzees. Nat Med. 1995; 1:1009–1016. [PubMed: 7489355]

3. DAHL LK, HEINE M, TASSINARI L. Effects of chronic excess salt ingestion. Evidence that genetic factors play an important role in susceptibility to experimental hypertension. J Exp Med. 1962; 115:1173–1190. [PubMed: 13883089]

4. Chen PY, Sanders PW. L-arginine abrogates salt-sensitive hypertension in Dahl/Rapp rats. J Clin Invest. 1991; 88:1559–1567. [PubMed: 1658045]

5. Bragulat E, de la SA, Antonio MT, Coca A. Endothelial dysfunction in salt-sensitive essential hypertension. Hypertension. 2001; 37:444–448. [PubMed: 11230316]

6. Bech JN, Nielsen CB, Ivarsen P, Jensen KT, Pedersen EB. Dietary sodium affects systemic and renal hemodynamic response to NO inhibition in healthy humans. Am J Physiol. 1998; 274:F914–F923. [PubMed: 9612329]

7. Ying WZ, Sanders PW. The interrelationship between TGF-beta1 and nitric oxide is altered in salt-sensitive hypertension. Am J Physiol Renal Physiol. 2003; 285:F902–F908. [PubMed: 12865256]

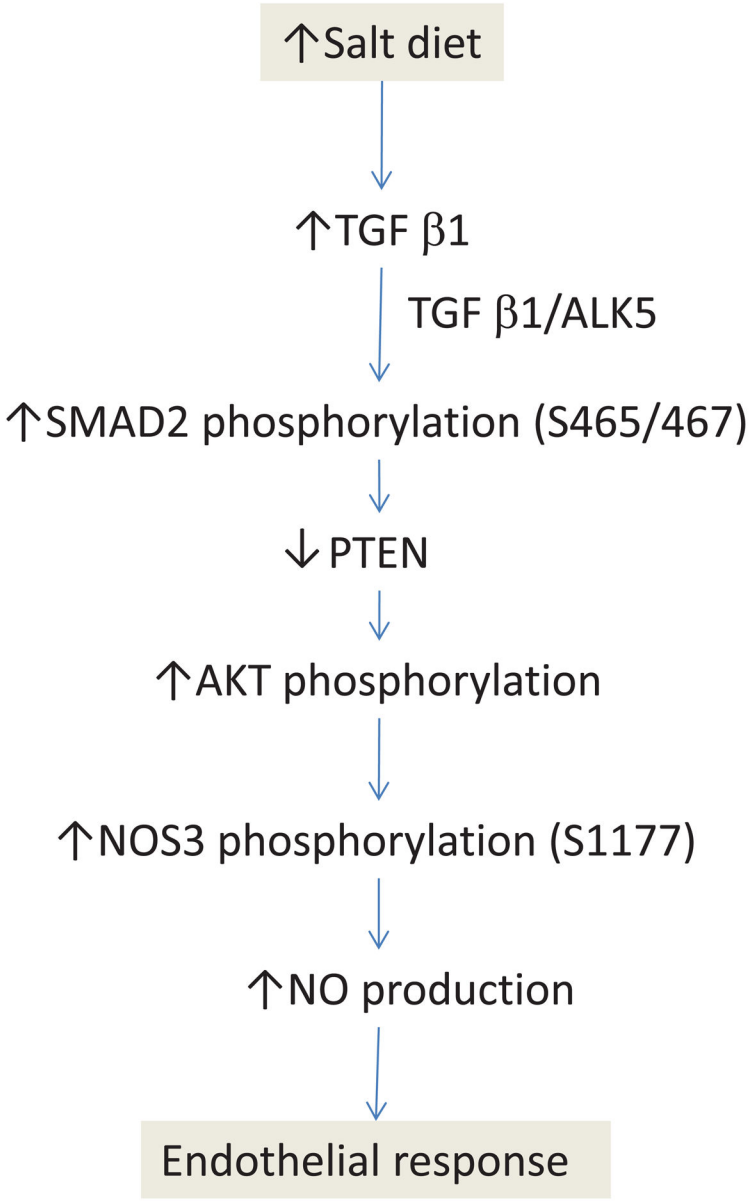

Figure.

Figure shows the endothelial response to a high salt diet. Transforming growth factor β1 is increased in response to a high salt diet that triggers downstream phosphorylation of SMAD2 (see text for discussion). Activation of this pathway triggers a decrease in Phosphatase and tensin homologue deleted on chromosome 10 (PTEN) that in turn permits an increase in Akt activity and phosphorylation of the endothelial isoform of nitric oxide synthase. Endothelial nitric oxide restores endothelial function. Problems in this transduction pathway can lead to an impaired response to a high salt diet and endothelial dysfunction.