Vascular consequences of dietary salt intake

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Sanders PW. Vascular consequences of dietary salt intake. Am J Physiol Renal Physiol 297: F237–F243, 2009. First published April 1, 2009; doi:10.1152/ajprenal.00027.2009.—Animal and human studies support an untoward effect of excess dietary NaCl (salt) intake on cardiovascular and renal function and life span. Recent work has promoted the concept that the endothelium, in particular, reacts to changes in dietary salt intake through a complex series of events that are independent of blood pressure and the renin-angiotensin-aldosterone axis. The cellular signaling events culminate in the intravascular production of transforming growth factor-β (TGF-β) and nitric oxide in response to increased salt intake. Plasticity of the endothelium is integral in the vascular remodeling consequences associated with excess salt intake, because nitric oxide serves as a negative regulator of TGF-β production. Impairment of nitric oxide production, such as occurs with endothelial dysfunction in a variety of disease states, results in unopposed excess vascular TGF-β production, which promotes reduced vascular compliance and augmented peripheral arterial constriction and hypertension. Persistent alterations in vascular function promote the increase in cardiovascular events and reductions in renal function that reduce life span during increased salt intake.

transformation growth factor-β; nitric oxide; vascular remodeling; arterial compliance

INVESTIGATORS STUDYING DIETARY NaCl (salt) intake observed more than a half century ago a dose-dependent decrease in survival of rats fed a standard diet containing increasing amounts of salt (Fig. 1) (38). The authors suggested that a high-salt diet increased mortality by accelerating atherosclerosis, arteriolosclerosis, and renal parenchymal damage. These outcomes were associated with changes in blood pressure, which increased in a dose-dependent fashion after 9 mo on the diets, although some animals were remarkably resistant to the hypertensive response to salt in the diet. This dramatic illustration of the effect of excess salt intake on mammalian cardiovascular and renal physiology has not been stressed, but recent developments have revitalized these original findings. With emphasis on the role of the renin-angiotensin-aldosterone system (RAAS) in cardiovascular disease states, an early concern was that salt restriction in the general population promotes significant, but small, decreases in systolic and diastolic blood pressure while producing a physiological, but large (>300%), increase in circulating renin and aldosterone levels (31). These issues were resolved when Cook et al. (11) performed a large prospective clinical trial and showed that, despite the small reductions in systolic (1.7 mmHg) and diastolic (0.8 mmHg) blood pressure, chronic salt restriction to ~2–2.6 g/day promoted cardiovascular health and reduced cardiovascular event rate by 25% in the intervention group.

In contrast to the physiological upregulation of the RAAS during low salt intake, pathological changes in the RAAS, as well as other genetic and acquired disorders that facilitate sodium retention (51), promote salt sensitivity, which accentuates the alterations in cardiovascular/renal function induced by salt excess (39, 63). An excellent example of the consequence of excess salt intake on end-organ damage in the setting of salt sensitivity induced by dysfunction of the RAAS comes from the study by Pimenta et al. (46), who observed that proteinuria correlated with urinary sodium excretion rates in patients with resistant hypertension and pathological aldosterone excess, but not in subjects with normal urinary aldosterone levels. The intent of this review is to delineate how excess salt intake alters cardiovascular function through mechanisms that involve, in particular, transforming growth factor (TGF)-β1 and nitric oxide (NO).

DIETARY SALT INCREASES PRODUCTION OF TGF-β

After the discovery of the initial member of the family of TGFs in 1980 (47), these important molecules have been shown to have complex effects on organ development and cell growth and differentiation, but they are particularly important in the expression of extracellular matrix proteins (49). Numerous studies have shown that these fibrogenic or prosclerotic growth factors participate integrally in vascular and renal fibrosis in a variety of disease states (1, 7, 13, 33, 54, 65, 66, 76, 77). Mammals express three isoforms of TGF-β: TGF-β1, -β2, and -β3. Although some differences exist, most evidence supports similar functions among these three TGF-β isoforms, with TGF-β1 considered the most important mammalian isoform. TGF-β1 is synthesized by many cell types, including endothelium, and is secreted as a latent dimeric ~75-kDa protein complex (3, 18). A latency-associated peptide is cleaved from the active TGF-β molecule by the enzyme furin, during intracellular processing, but remains noncovalently complexed to the mature peptide after secretion.
latent TGF-β-binding proteins, which are members of the fibrillin/lateral TGF-β-binding protein family, bind this complex and direct it to the adjacent interstitium. Once in the extracellular space, removal of latent TGF-β frees the mature, 24-kDa biologically active form of TGF-β (3). Thus endothelium-derived TGF-β is typically a locally acting molecule with autocrine and paracrine actions on neighboring endothelium and vascular smooth muscle. Although there are several known mechanisms of activation of TGF-β (3, 48), thrombospondin-1 appears to be a major regulatory factor involved in TGF-β activation following secretion by endothelial cells (53) and in vivo in a model of mesangial proliferative glomerulonephritis (14).

TGF-β is involved in blood pressure regulation. Elastin microfibril interface-located protein 1 (Emilin1), a glycoprotein expressed in the vascular tree, binds the TGF-β precursor and prevents processing by furin. EMILIN1 knockout mice, therefore, display increased TGF-β1 signaling in the vessel wall. These animals develop peripheral vasoconstriction and arterial hypertension, which were prevented by inactivation of one TGFβ1 allele (75). In another study, parenteral administration of an anti-TGF-β antibody to Dahl salt-sensitive rats every other day for 2 wk significantly reduced blood pressure and the associated proteinuria, glomerulosclerosis, and interstitial and vascular fibrosis observed in this model of salt-sensitive hypertension (13).

Vascular pathology encompasses remodeling not only of resistance vessels, but also compliance vessels. Arterial stiffness, an important determinant of systolic blood pressure, may also stimulate hypertrophy and remodeling of the microcirculation. The effect of reduced vascular compliance on the microcirculation is dramatic in the brain and kidney, two organs that are perfused at high flow rates and pulsatile pressures (43). Isolated systolic hypertension is also stimulated in resistance vessels, but also compliance vessels. Arterial stiffness, an important determinant of systolic blood pressure, may also stimulate hypertrophy and remodeling of the microcirculation. The effect of reduced vascular compliance on the microcirculation is dramatic in the brain and kidney, two organs that are perfused at high flow rates and pulsatile pressures (43). Isolated systolic hypertension is also stimulated in resistance vessels, but also compliance vessels.

Excess dietary salt intake increases vascular collagen deposition and TGF-β1 production. After the studies by Meneely and Ball (38), Tobian and Hanlon (58) suggested that a high-salt diet produced arterial lesions without increasing blood pressure. Deoxycorticosterone acetate and 1% salt were administered to uninephrectomized Dahl salt-resistant rats for 6 wk; then the animals were allowed to recover for 4 mo, during which deoxycorticosterone acetate and the salt additive were not administered. Determination of blood pressure at the end of the recovery period confirmed that the rats were hypertensive. The animals were then fed a diet that contained 0.3% or 8.0% NaCl. Although no further increases in blood pressure were observed, animals fed the high-salt diet had a very high mortality rate, which was attributed to cerebrovascular accidents (58). Yu and associates (74) demonstrated collagen deposition in the arteries, arterioles, glomeruli, and interstitium of the hearts and kidneys of normotensive (Wistar-Kyoto) and spontaneously hypertensive rats fed an 8.0% salt diet; upregulation of TGF-β1 mRNA was observed in the kidney and heart during administration of the high-salt diet in both strains. An 8.0% NaCl diet also increased albuminuria and accelerated progression of renal failure in a rodent model of chronic allograft nephropathy (52).

A major interest of our laboratory has been, in general, the effect of salt intake on endothelial cell function and, in particular, TGF-β1. Initial studies used Sprague-Dawley (SD) rats, in which blood pressure did not increase during the period of observation, to demonstrate that expression of all three mammalian TGF-β isoforms increased in cortical tissue of rats fed a formulated diet containing 8.0% NaCl, compared with tissue from rats maintained on a 0.3% NaCl diet (69). Increased of extracellular matrix proteins (28) and inhibiting activity of those metalloproteinases involved in collagen degradation and remodeling (40).

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expression was observed 1 day after institution of the diet and persisted over the 15 days of the experiment. Although serum levels of TGF-β1 did not change, urinary excretion of TGF-β1 increased on the 8.0% NaCl diet, indicating an intrarenal generation of TGF-β1. Administration of furosemide (5 mg/kg) and chlorothiazide (4 mg/kg) to rats maintained on the high-salt diet did not diminish the intrarenal generation of TGF-β1 (Fig. 2). Additional studies confirmed that an increase in salt intake increased the production of total and active TGF-β1 throughout the arterial tree, including the aorta (67), intrarenal arteries and arterioles (unpublished observations), and glomeruli (69). Removal of the aortic endothelium confirmed that this was the source of augmented TGF-β1 production during increased salt intake. Total and active TGF-β1 increased in the SD rats fed the high-salt diet.

**ENDOTHelial CELL SIGNALING MECHANISMS AND DIETARY SALT**

Hemodynamic forces are strong activators of the endothelium and modulate expression of numerous genes (23). By virtue of the location and response to shear stress, endothelial cells serve as biomechanical sensors that detect and respond to changes in blood flow (15), such as the change that occurs after expansion of blood volume when dietary salt is increased. Exposure of bovine aortic endothelial cells in culture to shear promoted TGFBI gene transcription and the release of biologically active TGF-β; these effects were inhibited by addition of tetraethylammonium (42). An increase in shear induced by creation of an aortocaval fistula produced an acute increase in the expression of TGF-β1 and β3 in the aorta (64). Endothelium-dependent NO production induced by flow is also inhibited by tetraethylammonium and charybdoxotin (12).

Consistent with a shear effect, the initial event that stimulates TGF-β1 production by aortic ring and glomerular preparations from SD rats fed a high-salt diet was the opening of a tetraethylammonium-sensitive potassium channel (67, 69). Downstream of the cell surface events, shear force activates the p38 MAPK (5, 29, 36) and p42/44 MAPK (29, 60, 61) pathways in endothelial cells in culture. Dietary salt activated, in a dose-dependent fashion, p38 MAPK and p42/44 MAPK pathways in aortic ring segments and preparations of isolated glomeruli (68, 70). Intravenous injection of tetraethylammonium just before harvesting of the tissue inhibited the increase in aortic and glomerular p38 and p42/44 MAPK activities that occurred with the high salt intake (68, 70). Thus these MAPK pathways were downstream of the tetraethylammonium-inhibitable potassium channel. Nuclear accumulation of phosphorylated p38 MAPK and p42/44 MAPK in the endothelial cells lining the aorta of SD rats on the 8.0% NaCl diet suggested activation of nuclear transcription. Additional experiments identified increased amounts of phosphorylated (activated) nuclear transcription factors that included activating transcription factor-2, which is activated by p38 MAPK, and Elk-1, which is a substrate of p42/44 MAPK, in SD rats fed the high-salt diet (70). Selective MAPK inhibitors demonstrated the essential role of both MAPK pathways in salt-induced increases in expression of TGF-β1 in aorta and glomeruli of SD rats (68, 70, 71).

**Fig. 3. Working model of salt-induced endothelial cell activation.** A: introduction of shear force activates the endothelium by opening a tetraethylammonium-inhibitable potassium channel. B: Pyk2 is autophosphorylated at Y402 and activated. C: c-Src is recruited and activated by Pyk2. Activity of Pyk2 is increased by c-Src-mediated phosphorylation at Y579/580 in the kinase domain. D: phosphatidylinositol 3-kinase (PI3K) is recruited to the complex, permitting activation of Akt and calcium-independent activation of nitric oxide synthase isoform 3 (NOS3) by phosphorylation at S1176 in rats. This complex also activates p38 and p42/44 MAPK pathways, resulting in augmented endothelial cell production of TGF-β1.
Recent studies focused on salt-induced activation of proline-rich tyrosine kinase 2 (Pyk2, also known as RAFTK, CAK-β, and CADTK), which is a member of the focal adhesion protein tyrosine kinase family (4). Pyk2 is activated by extracellular stress signals, such as shear stress (57), but also by G protein-coupled receptors, such as the angiotensin type 1 (AT1) receptor (4, 27). Increasing dietary salt intake by 0.3% and 8.0% NaCl induced a dose-dependent increase in activation of endothelial Pyk2, which recruited and activated c-Src (73). Complex formation between Pyk2 and c-Src was necessary to activate p38 and p42/44 MAPK and generate TGF-β1 (71). The combined data fit a working hypothesis that dietary salt intake affects endothelial cell activity through shear force.

ROLE OF NO

NO production becomes a central feature in the vascular response to salt intake. Increased salt intake increased NO production in SD and Dahl/Rapp salt-resistant (R) rats (8, 9, 17, 55) and healthy humans (6). Bech et al. (6) showed that increased dietary salt intake increased renal plasma flow and glomerular filtration rate and, further, demonstrated that the associated increase in NO production was integrally involved in this renal hemodynamic response as well as sodium excretion. Although the source of the increase in NO could not be determined in that study, the endothelium was the vascular source of the salt-induced augmented NO production in rats (Fig. 4) (67). Increased NO production promotes vasorelaxation of the afferent arteriole (30), augments glomerular filtration rate, and improves the pressure-natriuresis curve, facilitating salt excretion (16, 45). Inhibition of NO produces salt retention and salt-sensitive hypertension (59) and, if protracted, results in renal injury, particularly if the animals are maintained on a high-salt diet (22).

In addition to the hemodynamic effects, NO also modulates salt-induced TGF-β1 production. Glomerular and vascular ring preparations incubated with Nω-nitro-L-arginine methyl ester produced increased amounts of TGF-β1, whereas incubation with an NO donor, (±)-(E)-4-ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexenamide, decreased production of TGF-β1 (71). Thus NO limits the production of TGF-β1. The mechanism was independent of blood pressure and flow, since the experiments were performed in vitro. The proposed signaling mechanism of increased NO production is that shear forces generated by increased salt intake stimulate Akt-mediated phosphorylation of NO synthase isofrom 3 (NOS3) at amino acid residue 1,176 in rats. Activation of Akt occurred with the formation of a Pyk2-c-Src-phosphatidylinositol 3-kinase complex (72), and the findings are consistent with the known modulatory effect of Pyk2 on NOS3 activity in other conditions (37). These data are also consistent with the original description of shear-induced phosphorylation of NOS3 by Akt in endothelial cells (19) and with experiments that used isolated aortic ring segments (56). Interestingly, Soucy et al. (56) showed that aging promoted a decrease in flow-mediated activation of Akt and NO production and an associated increase in pulse-wave velocity compared with vascular tissue from young animals. Mice lacking NOS3 demonstrated higher mean pulse-wave velocities than wild-type mice. The authors suggested that impaired Akt-mediated NOS3 activation contributes to age-associated vascular stiffness (56).

During increased salt intake, NO production serves as a countervailing influence that mitigates the consequences of production of TGF-β1. Conceivably, endothelial dysfunction associated with aging promotes vascular stiffness from unop-
posed TGF-β1 production; this effect would be enhanced by the addition of an increased-salt diet, which stimulates intravascular production of TGF-β1 (73). Limiting salt intake might therefore improve arterial stiffness, a risk factor associated with cardiovascular events (35). In a double-blind, placebo-controlled, crossover study of older adults, a low salt intake increased carotid arterial compliance by 27% by the end of the 1st wk, and the improvement stabilized at 46% by the 2nd wk (24). These findings suggest that although conduit artery compliance is a function of aging, it is not irreversible, and decreased salt intake improves arterial stiffness.

NO production regulates blood pressure not only through vasorelaxation of resistance vessels but also by limitation of TGF-β1 production. The Dahl/Rapp salt-sensitive (S) rat strain rapidly develops hypertension and severe renal failure related to tissue hypoxia from a progressive arteriopathic process involving the interlobular arteries and preglomerular arterioles (8–10, 62). S rats manifest impaired NO production that is exacerbated by increased salt intake (8, 9). Vascular and glomerular production of active TGF-β1 and NO metabolites, nitrite and nitrate, were directly correlated in R and S rats, but production of TGF-β1 was increased in prehypertensive S rats, and differences in endothelial production of TGF-β1 and NO between S and R rats were further exaggerated with institution of the increased-salt diet (71). Along with the demonstrated antihypertensive action of anti-TGF-β in S rats (13), the augmented vascular and glomerular production of TGF-β1, exacerbated by impaired NO, may contribute integrally to the development of hypertension and hypertensive nephrosclerosis in this salt-sensitive strain.

In addition to aging, there are other factors that promote endothelial dysfunction. Evidence supports a direct effect of salt intake on endothelial function mediated through plasma sodium concentration. Endothelial cells in culture stiffened and produced less shear-induced NO when the medium concentration of sodium increased from 135 to 145 meq/l. Altered endothelial cell function from reduction of medium sodium concentration was observed only in the presence of aldosterone (41). However, because reduction of salt intake to very low levels (10 mmol/day) produced a small, but significant, reduction in plasma sodium concentration (~3 meq/l) compared with values obtained from the same patients on a 350 mmol/day salt diet (26), the overall significance of the in vitro studies is uncertain. Other disease states, including hypertension per se, promote oxidative stress and inflammation, which promote endothelial dysfunction and diminished production of NO (2, 25, 34, 50).

CONCLUSIONS

Dietary salt intake promotes endothelial cell production of TGF-β1 and NO (Fig. 5). NO release promotes vascular relaxation and inhibits TGF-β1 production. When NO production is impaired, such as with aging, hypertension, and other systemic diseases, unopposed excess vascular TGF-β1 production results in reduced vascular compliance and augmented peripheral arterial constriction and hypertension. Cerebrovascular and renovascular diseases are likely the proximate causes of reduced life span associated with excess salt intake. Meloney and Ball (38) reported that “salt is rough on rats,” and recent findings support adverse effects of salt on the vascular biology of humans as well. The obvious answer might be to simply reduce the content of salt in the diet, as demonstrated by Cook et al. (11). However, exactly how a low-salt diet promotes cardiovascular health when circulating angiotensin II levels are increased is a conundrum, particularly since angiotensin II can stimulate the production of TGF-β in vitro (32). TGF-β production in vivo is not increased with salt restriction. Diminished tissue responsiveness to angiotensin II seems an unlikely explanation, since adrenal production of aldosterone is similarly increased by a low-salt diet, although vascular expression of the AT1 receptor in response to changes in dietary salt intake has not been explored. Finally, another interesting observation from these early research pioneers is that increased dietary potassium intake exerted a protective action that diminished the mortality rate associated with excess salt intake in rats (38). Although the mechanism is forthcoming, these studies provide support for additional experiments that examine the mitigating role of dietary potassium in salt intake.

REFERENCES


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