Role of the Vascular Wall in Sodium Homeostasis and Salt Sensitivity

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ABSTRACT

Excessive sodium intake is associated with both hypertension and an increased risk of cardiovascular events, presumably because of an increase in extracellular volume. The extent to which sodium intake affects extracellular volume and BP varies considerably among individuals, discriminating subjects who are salt-sensitive from those who are salt-resistant. Recent experiments have shown that, other than regulation by the kidney, sodium homeostasis is also regulated by negatively charged glycosaminoglycans in the skin interstitium, where sodium is bound to glycosaminoglycans without commensurate effects on extracellular volume. The endothelial surface layer is a dynamic layer on the luminal side of the endothelium that is in continuous exchange with flowing blood. Because negatively charged glycosaminoglycans are abundantly present in this layer, it may act as an intravascular buffer compartment that allows sodium to be transiently stored. This review focuses on the putative role of the endothelial surface layer as a contributor to salt sensitivity, the consequences of a perturbed endothelial surface layer on sodium homeostasis, and the endothelial surface layer as a possible target for the treatment of hypertension and an expanded extracellular volume.


In Western society, average daily intake of salt is 8–12 g, thereby greatly exceeding the recommended amount by the World Health Organization of 5 g daily.1,2 This recommendation is on the basis of the observation that dietary salt intake exceeding 5 g/d, which is equivalent to 2 g or 85 mmol sodium, is associated with hypertension and increased cardiovascular risk in many cohort studies.3,4 Other than negative effects on cardiovascular morbidity and mortality, high salt intake has also been related to intermediate end points for kidney damage, such as proteinuria, in both patients with CKD and the general population.5,6 Dietary salt restriction is, therefore, regarded as an important target for improvement of global health.4 For example, in the United States, it has been estimated that a reduction of dietary salt intake by 3 g/d would reduce annual health costs by $10–$24 billion.7

Generally, detrimental effects of excessive sodium intake have been linked to expansion of extracellular volume (ECV) and hypertension, which is evidenced by various observations that low sodium reduces BP in both normotensive individuals and individuals with hypertension.4,8 The increase in BP after dietary sodium excess is highly variable, with some individuals showing a relatively small increase, whereas large BP increases can be observed in others.9,10 It is likely that these individual variations in salt sensitivity differentially affect cardiovascular and renal risk and may also explain the inconsistent results from population studies investigating the relation between sodium intake and cardiovascular risk.11

According to Guyton’s pressure-natriuresis curve, the kidney regulates long-term BP by altering renal sodium excretion in response to variations in sodium intake. When renal sodium excretion capacity is limited by intrarenal or extrarenal factors, this will result in an increase in BP, which in turn, increases renal sodium excretion at the expense of a higher BP (i.e., salt sensitivity).12 The pathophysiology of the differential BP response to a salt load has not yet been fully elucidated.13 Recent studies have shown that, other than mechanisms that directly or indirectly influence renal sodium excretion, an extrarenal compartment exists in the skin interstitium, where glycosaminoglycans (GAGs) bind and inactivate sodium (i.e., nonosmotic sodium storage).14 The endothelial surface layer (ESL), also containing these GAGs, may be another compartment involved in nonosmotic storage of sodium with important implications for ECV and BP regulation.
NONOSMOTIC SODIUM STORAGE

On the basis of the intracellular and extracellular sodium concentrations and the distribution of total body water (42 L in an average 70-kg man), it is classically thought that about 1960 mmol sodium (140 mmol/L; 14 L) is present in the extracellular compartment and 336 mmol sodium (12 mmol/L; 28 L) is present in the intracellular compartment. The intravascular compartment representing one third of the ECV contains approximately 653 mmol sodium in 4.7 L. The notion that a significant proportion of sodium is nonosmotically stored has dramatically changed this view. The evidence for nonosmotic sodium storage has come from space simulation programs that have monitored electrolyte intake and excretion increased to 2973–7324 mmol over 135 days of normal dietary sodium intake (160 mmol sodium/d) without any change in total body water. A second study in individuals on a long-term stable sodium diet showed changes in total body sodium (–200 to +200 mmol) that were not related to changes in ECV or body weight, advocating the presence of a clinically relevant buffer where sodium can be nonosmotically stored.16 The capacity for nonosmotic sodium storage has been shown for various tissues, such as skin, cartilage, bone, and muscle.14,17–20 Studies using 23Na magnetic resonance imaging and conventional 1H magnetic resonance imaging have revealed that, in both patients with primary hyperaldosteronism and patients with essential hypertension, considerable amounts of sodium are stored in muscle and skin without commensurate water retention.19,20

Experiments in both mice and rats have identified that GAGs in the skin interstitium are responsible for sodium storage.14,17 GAGs are large, negatively charged linear polymers consisting of disaccharide unit repeats. Specific combinations of these repeating units result in different types of GAGs, such as heparan sulfate, chondroitin sulfate, dermatan sulfate, keratan sulfate, and hyaluronan, which have reviewed elsewhere. The negative charge density of GAGs is determined by their sulfation grade.22 In rats, high dietary sodium intake has been shown to coincide with increased interstitial GAG content as well as increased polymerization and sulfation of these GAGs, with skin sodium concentrations (180–190 mmol/L) increasing to values far exceeding plasma sodium concentrations under these conditions.14,17 Because these high extracellular sodium concentrations were not accompanied by extracellular water retention, it was proven that a substantial part of the measured sodium has to be stored osmotically inactive. The concept that GAGs have the capacity to bind and osmotically inactivate sodium is not new. In 1957, Farber et al.23 dialyzed sodium chondroitin sulfate against sodium chloride at various concentrations in an in vitro experiment. After 24 hours, higher sodium concentrations were consistently found in the chondroitin sulfate compartment compared with sodium concentrations outside this compartment, whereas chloride was equally distributed among both compartments.23 These results indicate that a part of the sodium present in the chondroitin sulfate solution was not ionized but inactivated by this GAG, because it did not exert the expected osmotic effect.23 From an evolutionary point of view, genes involved in GAG sulfation are highly conserved.24 GAG sulfation degree does not increase in higher animals. In general, aquatic species contain a more structural variety in their GAGs than terrestrial animals, and the degree of sulfation increases as a function of the salt content of the environment of the organism.24 Because interactions between components of the extracellular matrix seem to occur at higher salt concentrations in marine invertebrates than vertebrates, GAGs with increased charge density may be needed for keeping the intracellular environment stable, indicating that the more a subject is exposed to sodium, the more sulfated GAGs may be required.24,25 In humans, highly sulfated heparan sulfates are mainly found in tissues having a barrier function, including skin, lung, intestine, and endothelium.

ESL FUNCTION

The ESL is a dynamic layer on the luminal side of the endothelial cell that is in continuous exchange with flowing blood. It comprises a network of glycoproteins, adsorbed plasma proteins, and proteoglycans to which GAG chains are attached. Heparan sulfate GAGs are most prominent on endothelial cells followed by chondroitin sulfate and hyaluronan GAGs. ESL composition and volume depend on the local microenvironment and are actively regulated by endothelial cells.21 The ESL is instrumental in regulating vascular permeability and hemostasis and possesses antiatherogenic and anti-inflammatory properties.21 Moreover, the ESL is an important mediator in shear-induced nitric oxide (NO) production.21

Other than these features, the highly sulfated negatively charged GAGs within the ESL may have sodium-binding properties representing a potential buffering zone for circulating sodium. In contrast to the skin interstitium, the ESL is in direct contact with plasma sodium and therefore, could function as a first sodium buffer before sodium enters the interstitium. The negative charges of the endothelial cell and ESL automatically attract ions of the opposite charge when they are located within an electrolyte solution, such as blood.26 Because sodium is the most abundant cation in circulating blood, sodium forms a so-called ion atmosphere around the endothelial cell and ESL. Considering the sodium-binding properties of GAGs, it is conceivable that the attracted sodium ions are bound and inactivated by GAGs in the ESL. This has been shown in vitro by 23Na nuclear magnetic resonance experiments that have shown that sodium reversibly binds to GAGs in the ESL under flow.26 Because of abundant presence of highly sulfated, negatively charged GAGs, the ESL may have a significant role in sodium homeostasis. At present, the sodium-binding capacity of the ESL is not known. The negative charge of the entire vascular ESL in humans has been estimated to be able to inactivate about 30 mmol sodium.27 These calculations are on the basis of in vitro experiments and most likely
underestimate ESL dimensions, because 7–30 times larger ESL volumes have been reported in vivo, suggesting that, under normal physiologic conditions, the ESL may be able to store significant amounts of sodium.28,29

Current methods to measure ESL dimension in humans comprise measurement of shedding products in plasma, noninvasive side stream dark-field or orthogonal polarization spectral imaging of the sublingual microcirculation, in which the erythrocyte–endothelium gap is estimated, and a tracer dilution technique, in which circulating blood volume is compared with the distribution volume of an ESL-permeable tracer.30–32

Great variability in systemic ESL volume has been reported between different medical conditions, regardless of how ESL was estimated.33–35 Mean systemic ESL volume in healthy individuals was shown to be 1.5–1.7 L, whereas much lower systemic ESL volumes were found in treated (1.1 L) and untreated (0.8 L) patients with heterozygous familial hypercholesterolemia and normoalbuminuric (0.8 L) and microalbuminuric (0.2 L) patients with type 1 diabetes.33,34 Elevated shedding products, reflecting ESL breakdown, have been found in patients with severe sepsis, CKD, or ESRD, patients on dialysis, patients after major vascular surgery, and patients during acute or chronic hyperglycemia.31,33,35–40 Most of these conditions associated with a perturbed ESL are also characterized by an expanded ECV, higher BP, or both, suggesting that variability in sodium homeostasis and salt sensitivity may be related to the quality of the ESL, in which endothelial GAGs act as an intravascular buffer compartment for sodium.

Considering the large ESL volume, it seems likely that, next to the skin interstitium, nonosmotic sodium storage by endothelial GAGs is a clinically relevant sodium buffer. In patients on hemodialysis, for example, increased plasma syndecan-1 levels, reflecting ESL breakdown, have been associated with an increased need for ultrafiltration, advocating that loss of ESL sodium buffer capacity is clinically relevant in patients prone to volume overload.41 In addition, in patients with type 1 diabetes characterized by a decreased ESL volume, BP was inversely associated with ESL volume, which suggests a possible association between ESL volume and BP regulation.33

Although GAGs in the ESL seem to relate to sodium homeostasis, so far, few studies have investigated the direct effects of sodium on ESL volume and function. Most of the observations have come from experiments that have studied the interaction between resuscitation fluids and vascular barrier function. After acute volume loading, ESL volume has been shown to decrease considerably independent of the colloidal resuscitation fluid used (5% albumin or 6% hydroxyethylstarch; both containing 0.9% NaCl).32 For example, after 5% albumin infusion (20 ml/kg; 220 mmol sodium in a 70-kg person), absolute ESL volume decreased by 47%.42 These data may suggest a direct detrimental effect of acute sodium loading on ESL volume, which has been supported by in vitro data reporting that high sodium concentrations decrease ESL volume as measured by atomic force microscopy.43 However, expansion of intravascular volume and subsequent ESL compaction may serve as an alternative explanation for the reduction in ESL volume. Because these observations are on the basis of acute sodium loading by using colloids, they may not reflect ESL changes that might occur during chronic high dietary sodium intake.

![Figure 1. The putative effects of the ESL on sodium homeostasis. Negatively charged GAGs have been shown to be able to osmotically inactivate circulating sodium ions.14,23 These GAGs are abundantly present in the ESL, where they may function as a first buffer that inactivates consumed sodium. In addition, an intact ESL has been shown to control EnNaC-mediated sodium transport into the endothelial cell and be crucial for shear stress-mediated NO production.44–46 When the ESL is perturbed and its buffer and barrier functions are lost, an increased amount of osmotically active sodium is located in the vascular lumen, which can lead to water retention. Moreover, shear stress-mediated NO production will be diminished, and EnNaC-mediated sodium transport into the endothelial cell will increase, subsequently reducing NO production.47 An increase in dietary sodium intake may, therefore, result in a BP increase when the ESL is perturbed, whereas an intact ESL prevents the BP from rising. SMC, smooth muscle cell.](http://www.jasn.org)
Changes in ESL characteristics may lead to changes in endothelial cell function. Enzymatic removal of GAGs in the ESL, for example, has been shown to significantly decrease shear-induced NO production. Apart from regulating mechanotransduction, the ESL has been shown to determine NO availability by mediating sodium transport into the endothelial cell. After discovery of the epithelial sodium channel on the endothelial luminal surface (EnNaC) next to its known presence on the apical plasma membrane of epithelia, it was shown that this EnNaC regulates endothelial nanomechanics and subsequently affects NO production. The stiffness subsequently affects NO production. 

Regulates endothelial nanomechanics and of epithelia, it was shown that this EnNaC 

By enhancing sodium influx, the EnNaC increases mechanical stiffness of the endothelial cellular cortex. The stiffness of this 50- to 100-nm layer, which mainly consists of actin filaments, subsequently modulates endothelial NO synthase activity and NO production, where an increasing stiffness attenuates NO production. The density of EnNaCs on the endothelial surface is regulated by aldosterone and plasma sodium concentration. A rise in plasma sodium concentration increases EnNaC density, which in turn, increases sodium uptake, stiffens the endothelial cellular cortex, and subsequently, leads to diminished NO production. An increase in sodium delivery to the endothelial cell as a result of an increase in sodium intake could, therefore, lead to an increase in vascular tone. These experimental findings are consistent with studies in humans, in which dietary sodium restriction has been shown to improve macrovascular and microvascular endothelial function by an enhanced bioavailability of NO directly induced by the low sodium diet. By covering the endothelial cells, an intact ESL may be pivotal to control EnNaC-mediated sodium transport into endothelial cells. Enzymatic removal of the ESL has been shown to facilitate EnNaC-mediated sodium transport into the endothelial cells, which led to increased endothelial stiffness. These results indicate that an intact ESL functions as a barrier to control EnNaC-mediated sodium transport. 

On the basis of the data as discussed above, the ESL seems to affect sodium homeostasis by functioning as an intravascular buffer for sodium as well as a barrier protecting the endothelial cell against EnNaC-mediated sodium uptake (Figure 1). An intact ESL having sufficient buffering function would, therefore, be able to transiently store osmotically active sodium, whereas a perturbed ESL cannot. In the latter condition, a sodium load would result in water retention and a rise in BP because of an increase in osmotically active sodium (Figure 2). A decrease in shear-mediated NO production will presumably allow an additional BP increase.

**ESL MODULATION**

Irrespective of the presence of salt sensitivity, dietary sodium reduction represents the cornerstone in the treatment of hypertension and expanded ECV. Sodium restriction has been shown to reduce BP and potentiate antihypertensive treatment, including renin-angiotensin system blockers and diuretics, and it has even shown similar BP reduction as a single-drug treatment. Moreover, because a small increase in plasma sodium (1.5–3.0 mmol) already can be achieved with an increased dietary sodium intake for 4–14 days, dietary sodium restriction is a simple way to prevent sodium-induced changes of endothelial function.

Beyond the beneficial effects of dietary sodium restriction and diuretic therapy, preservation and restoration of the ESL seem to be an interesting new target for treatment in cardiovascular and renal diseases. In this respect, sulodexide, a highly purified mixture of ESL constituents containing 20% dermatan sulfate and 80% heparan sulfate GAGs, is of interest. The relative bioavailability of oral sulodexide (40%–60%) and its relatively long elimination half-life (19–26 hours) make it suitable for oral administration. In patients with diabetes, sulodexide has been shown to restore ESL dimension. In addition, sulodexide treatment has been shown to improve albuminuria in patients with types 1 and 2 diabetes in several small studies. Various mechanisms that could be responsible for an increase in ESL volume and a decrease in albuminuria have been described. For instance, it has been shown that sulodexide increases synthesis and sulfation of heparan sulfates. Both mechanisms are thought to attenuate glomerular capillary

![Figure 2. Proposed influence of a perturbed ESL on the relation between dietary salt intake and BP. According to Guyton’s pressure–natriuresis curve, the kidneys regulate long-term BP by altering renal sodium excretion. When renal sodium excretion capacity is limited by intrarenal or extrarenal factors, this will result in an increase in BP, which in turn, increases renal sodium excretion capacity at the expense of an increase in BP (i.e., salt sensitivity). Other than factors that limit renal sodium excretion, the ESL may also determine salt sensitivity. An intact ESL, containing many GAGs, may provide a first intravascular buffer that osmotically inactivates sodium before it results in water retention and increases BP. When the ESL is perturbed, sodium cannot be buffered in the ESL, and the curve between dietary salt intake and BP is expected to shift to the right.](image)
permeability for plasma proteins. However, in the randomized, double-blind, placebo-controlled, sulodexide macroalbuminuria trial that included 1248 patients, no additional renoprotective effect of sulodexide was seen when added to maximal renin-angiotensin system blocking therapy in patients with stages 3 and 4 CKD after a mean follow-up of 10 months.65 Interestingly, this trial showed a small but significant reduction in systolic BP after sulodexide treatment compared with placebo.65 BP-reducing effects have also been observed in a number of other randomized, controlled trials investigating long-term sulodexide administration (Table 1).58,60 In addition, a trial investigating effects of sulodexide on ESL reported that the observed drop in BP after sulodexide treatment (systolic/diastolic BP=−2/−3 mmHg) coincided with increased retinal and sublingual ESL dimension in patients with diabetes, whereas no BP reduction or ESL restoration was observed in control subjects.57 It is conceivable that the observed reduction in BP in these studies may have resulted from an increased non-osmotic sodium storage capacity after restoration of the ESL or by increased sulfation that also allows an increased amount of sodium to be buffered.57,63 An increase in shear-mediated NO production or preservation of endothelial function as a result of increased ESL dimensions may also have contributed to the BP changes induced by sulodexide. The potency of sulodexide to reduce BP is still difficult to estimate. Not all trials investigating long-term sulodexide administration reported BP values or BP reduction, and none of the studies were designed to specifically examine the effect of sulodexide on BP.59,66–68 Moreover, BP was already on target when sulodexide was added in most of the studies, and antihypertensive drugs were not actively controlled.58,66–68 Because the largest BP-lowering effect (mean placebo-subtracted BP reduction=−14.6/−8.3 mmHg) was observed in a crossover study in patients with diabetes and uncontrolled hypertension, sulodexide may especially lower BP in patients with hypertension and expanded ECV.60

In conclusion, new pathophysiologic concepts have arisen from the notion that sodium homeostasis and salt sensitivity seem to relate to not only the kidney, but also extrarenal factors—most intriguingly, the endothelium. This novel concept may provide alternative therapeutic targets in treatment of expanded ECV and hypertension in the future, but first, additional studies need to assess the extent to which changes in the sodium-buffering capacity of the ESL translate into changes in ECV, BP, and ultimately, cardiovascular and renal risk.

TABLE 1. Data of all randomized, placebo-controlled trials conducted that reported BP before and after treatment with sulodexide

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Mean Age (yr)</th>
<th>Sulodexide Dose (mg)</th>
<th>Duration (mo)</th>
<th>BP at Baseline</th>
<th>BP after Treatment</th>
<th>Placebo Subtracted BP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bang et al.68</td>
<td>77 Macroalbuminuric patients with IgA nephropathy treated with renin-angiotensin system inhibition</td>
<td>40</td>
<td>150</td>
<td>6</td>
<td>121.4/73.6</td>
<td>120.4/71.0</td>
<td>−0.6/−4.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>42</td>
<td>75</td>
<td></td>
<td>118.9/73.1</td>
<td>121.8/73.8</td>
<td>3.3/−0.7</td>
</tr>
<tr>
<td>Gambaro et al.58</td>
<td>223 Microalbuminuric and macroalbuminuric patients with types 1 and 2 diabetes</td>
<td>47</td>
<td>200</td>
<td>4</td>
<td>139.7/82.8</td>
<td>136.8/81.2</td>
<td>−1.6/−2.5</td>
</tr>
<tr>
<td>Heerspink et al.67</td>
<td>149 Microalbuminuric patients with type 2 diabetes treated with maximally dosed renin-angiotensin system inhibition</td>
<td>61</td>
<td>400</td>
<td>6</td>
<td>129/75</td>
<td>129/73</td>
<td>−1/0</td>
</tr>
<tr>
<td>Packham et al.65</td>
<td>1248 Macroalbuminuric patients with type 2 diabetes treated with maximally dosed renin-angiotensin system inhibition</td>
<td>62</td>
<td>200</td>
<td>10a</td>
<td>138.0/73.6</td>
<td>137.1/72.8</td>
<td>−2.4/−NA</td>
</tr>
<tr>
<td>Solini et al.60</td>
<td>12 Microalbuminuric and macroalbuminuric patients with diabetes and hypertension</td>
<td>52</td>
<td>100</td>
<td>4</td>
<td>155/81</td>
<td>143/75</td>
<td>−14.6/−8.3</td>
</tr>
</tbody>
</table>

NA, not available.
aBP measurements after 3 months.
bP=0.04 compared with placebo treatment.
cP=0.002 compared with placebo treatment.

REFERENCES


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DISCLOSURES

None.

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