Sodium: A Wolf in Sheep's Clothing

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Atomic force microscopy • Endothelial stiffness • Plasma potassium • Aldosterone • Spironolactone

Abstract

High blood pressure is the main cause of disease-related morbidity and mortality worldwide. It is virtually absent in populations that consume natural foods low in sodium. In other countries, however, where the individual intake of sodium is at least 10 times greater, the prevalence of arterial hypertension is about 40%. Vascular endothelium plays a central role in blood pressure regulation. In addition to the kidney, the vasculature is a major target for aldosterone where it controls nonexcitable sodium channels in the endothelium. High sodium channel expression/activity downregulates the release of nitric oxide (NO), and thus determines endothelial function. Mechanical cell stiffness is therefore the means whereby high sodium channel activity reduces NO release. We have found that small changes of plasma sodium, but only above 139 mM, and potassium above 4 mM regulate the stiffness of the submembrane actin web and thus control the shear stress-dependent activity of endothelial NO synthase, which lies directly beneath the cell membrane. Plasma sodium above 139 mM stiffens the actin web of the endothelial cell, and plasma potassium greater than 4 mM does the opposite. In conclusion, vascular endothelial cells are highly sensitive to changes in extracellular sodium and potassium but only within and above the normal range. This sensitivity may serve as a physiological feedback mechanism to regulate local blood flow. It becomes pathogenic,

however, when the concentration of plasma sodium or potassium changes towards and beyond the upper limits of the normal range.

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In cellular physiology, stiffness is the force necessary to compress a cell for a certain distance (piconewtons per nanometer). In life, force is applied to most tissues, particularly to vascular endothelium. Hemodynamic forces, generated by the heart's contraction, give rise to shear stress at the endothelial surface. It is inevitable, therefore, that the apical cell surface undergoes pulsatile reversible deformation. It is this mechanical stimulus which triggers the activity of endothelial nitric oxide synthase (eNOS) and the release of nitric oxide (NO) [1]. The NO diffuses to the adjacent vascular smooth muscle cells which relax leading to vasodilation. This regulatory mechanism distributes the blood in the organism according to its metabolic demands. Shear stress also causes a stiff (less deformable) cell to release less NO. Endothelial mechanical stiffness, therefore, is a key function in the control of local blood supply and presumably, arterial blood pressure.

Sodium

For more than three million years, primitive man consumed less than 1 g per day of sodium chloride [2]. About 8,000 years ago, with the advent of agriculture and farm-

Table 1. Extracellular sodium concentration and endothelial cell stiffness in vitro

Plasma sodium, mM	137	139	141	143	145	147
Increase in endothelial stiffness, %	0	+3 ± 1.3	+6 ± 1.8	+15 ± 2.4	+25 ± 2.8	+34 ± 3.6

Reference value is a sodium concentration of 137 mM. Values (±SEM) taken from Bussemaker et al. [20] and Fels et al. [21].

Fig. 1. Changes in endothelial cell stiffness in response to increasing sodium concentration. From Oberleithner et al. [8], with modifications.

ing, sodium chloride consumption increased to about 10 g per day. The main reason was to preserve foods such as bread and meat. Salt became precious because it allowed food to be stored for prolonged periods [3]. As life expectation is now close to 80 years, the harmful effects of high salt diets have become increasingly apparent. Hypertension, stroke, coronary heart disease and renal fibrosis are related to a high sodium intake [4]. Although the deleterious effects of a high sodium intake are obvious, the underlying mechanisms are still unclear. A high sodium intake causes fibrosis and inflammatory processes in the kidney and heart [5]. When dietary salt intake temporarily exceeds renal excretory capacity, the retained amount of sodium, stored in the space between cells, is increased, bound to extracellular organic material [6]. When sodium intake is raised in normal subjects and patients with hypertension, plasma sodium concentration increases by 1–4 mM [7]. It has therefore been postulated that changes in plasma sodium, per se, may influ-

ence blood pressure. Accordingly, the effect of such small changes in sodium concentration on endothelial function has been studied. It has been found that when extracellular sodium is raised, endothelial cells stiffen within minutes [8]. It is remarkable, however, that in striking contrast these cells are insensitive to sodium concentrations below 139 mM. Above this concentration, however, they are highly sensitive (fig. 1; table 1). This response of the endothelium to these changes in sodium concentration is strongly dependent on aldosterone. Inhibition of the cytosolic mineralocorticoid receptors by spironolactone (or eplerenone) prevents a rise in plasma sodium stiffening the endothelium, as does inhibition of the epithelial sodium channel (ENaC; present in the apical plasma membrane of endothelia) by amiloride [8]. These in vitro experiments may explain the protective action of these two pharmaceuticals on the cardiovascular system [9, 10]. The data strongly support the view that blood vessels and the heart, in addition to their susceptibility to the concentration of sodium, are also primary targets for diuretics, independent of their action on the kidney.

Potassium

In addition to the intake of sodium, the onset of hypertension is also controlled by the intake of potassium. Processed food products, in contrast to natural food, are rich in sodium and poor in potassium. It is generally agreed that low sodium high potassium diets, which have the least harmful effect on the cardiovascular system [11], may also have a beneficial effect on vigor and emotions such as depression and tension [12]. Potassium deficiency is difficult to detect for 98% of body potassium is intracellular. Plasma potassium, however, is controlled within narrow limits and is a sensitive indicator of a disturbance in potassium homeostasis. The effect of extracellular potassium on cultured vascular endothelial cells was therefore studied (table 2). It was found that extracellular potassium concentrations above 5 mM enlarge and soften endothelial cells [13]. In kidney disease, plasma potassi-

Table 2. Extracellular potassium concentration and endothelial cell stiffness in vitro

Plasma potassium, mM	4	4.5	5.0	5.5	6.0	6.5
Decrease in endothelial stiffness, %	0	-2 ± 1.3	-4 ± 1.8	-11 ± 2.4	-16 ± 2.8	-19 ± 3.6

Reference value is a potassium concentration of 4 mM. Values ($\pm$ SEM) taken from Oberleithner et al. [22].

um is often raised due to acidosis and the retention of potassium. In normal subjects, during exercise, though plasma potassium concentration remains normal, there may be a rise greater than 5 mM in muscle [14]. A similar rise occurs in the brain during increased neuronal activity [15].

The extent of potassium-induced endothelial cell softening depends on the concentration of extracellular sodium. In 'low' sodium conditions (sodium <140 mM), an increase in extracellular potassium from 4 to 6 mM reduces stiffness by 21%. In 'high' sodium conditions (sodium >140 mM), however, the decrease in stiffness induced by the same rise in potassium is only about 6% [13]. These observed interactions between extracellular sodium and potassium have been observed in vitro under well-controlled conditions. The situation may be different in vivo, but it should be kept in mind that mechanical stiffness (i.e. cell deformability or compliance) determines NO release.

Nitric Oxide

eNOS is located close to the inner surface of the apical cell membrane, and its expression and/or activity is regulated by various factors. eNOS is stimulated by increases in intracellular Ca^{2+} independent of the initial stimulus, e.g. bradykinin or shear stress. There is accumulating evidence that sodium and aldosterone control eNOS activity, possibly through ENaC-mediated sodium influx. On the other hand, inhibition of ENaC-mediated sodium influx by amiloride activates eNOS [16]. An increase in the intake of salt induces the intracellular production of asymmetrical dimethyl-L-arginine which is a competitive eNOS inhibitor [17], and increasing extracellular sodium within the physiological range downregulates eNOS expression and angiogenesis [18]. It is noticeable that (a) there is a negative correlation between mechanical stiffness and eNOS activity, (b) extracellular sodium concentration strongly determines stiffness and therefore eNOS function, and (c) extracellular potassium con-

centration influences eNOS activity and stiffness only at 'low' sodium concentrations.

A question remains: Does eNOS activity depend on cell stiffness or does cell stiffness depend on eNOS activity? In other words, does the cell deformability determine eNOS activity or does NO modify the mechanical compliance of the cell? This question was recently addressed in our laboratory using an experimental procedure that allowed the simultaneous measurement of hormone-induced change in stiffness and NO release. The results clearly show that mechanical stiffness determines NO release and not vice versa [19].

Outlook

The finding that endothelial cells modify their function in response to small changes in the extracellular concentration of sodium and potassium, only within and above the normal range, could be relevant to dialysis patients. These in vitro experiments suggest that the most favorable condition for the maintenance of normal blood pressure and the prevention of cardiovascular damage is to consume a diet which tends to keep plasma sodium and plasma aldosterone in the low-normal range and plasma potassium in the high-normal range.

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Disclosure Statement

There is no conflict of interest.

References

- 1 Rizzo V, McIntosh DP, Oh P, Schnitzer JE: In situ flow activates endothelial nitric oxide synthase in luminal caveolae of endothelium with rapid caveolin dissociation and calmodulin association. *J Biol Chem* 1998;273:34724–34729.
- 2 Meneton P, Jeunemaitre X, de Wardener HE, MacGregor GA: Links between dietary salt intake, renal salt handling, blood pressure, and cardiovascular diseases. *Physiol Rev* 2005;85:679–715.
- 3 Ritz E: Salt – friend or foe? *Nephrol Dial Transplant* 2006;21:2052–2056.
- 4 Adroge HJ, Madias NE: Sodium and potassium in the pathogenesis of hypertension. *N Engl J Med* 2007;356:1966–1978.
- 5 Sanders PW: Vascular consequences of dietary salt intake. *Am J Physiol Renal Physiol* 2009;297:F237–F243.
- 6 Titze J, Lang R, Ilies C, Schwind KH, Kirsch KA, Dietsch P, Luft FC, Hilgers KF: Osmotically inactive skin Na⁺ storage in rats. *Am J Physiol Renal Physiol* 2003;285:F1108–F1117.
- 7 de Wardener HE, He FJ, MacGregor GA: Plasma sodium and hypertension. *Kidney Int* 2004;66:2454–2466.
- 8 Oberleithner H, Riethmuller C, Schillers H, MacGregor GA, de Wardener HE, Hausberg M: Plasma sodium stiffens vascular endothelium and reduces nitric oxide release. *Proc Natl Acad Sci USA* 2007;104:16281–16286.
- 9 Pitt B, Remme W, Zannad F, Neaton J, Martinez F, Roniker B, Bittman R, Hurley S, Kleiman J, Gatlin M: Eplerenone, a selective aldosterone blocker, in patients with left ventricular dysfunction after myocardial infarction. *N Engl J Med* 2003;348:1309–1321.
- 10 Teiwe J, Toto RD: Epithelial sodium channel inhibition in cardiovascular disease a potential role for amiloride. *Am J Hypertens* 2007;20:109–117.
- 11 Haddy FJ, Vanhoutte PM, Feletou M: Role of potassium in regulating blood flow and blood pressure. *Am J Physiol Regul Integr Comp Physiol* 2006;290:R546–R552.
- 12 Torres SJ, Nowson CA, Worsley A: Dietary electrolytes are related to mood. *Br J Nutr* 2008;100:1038–1045.
- 13 Oberleithner H, Callies C, Kusche-Vihrog K, Schillers H, Shahin V, Riethmuller C, MacGregor GA, de Wardener HE: Potassium softens vascular endothelium and increases nitric oxide release. *Proc Natl Acad Sci USA* 2009;106:2829–2834.
- 14 Mohr M, Nordsborg N, Nielsen JJ, Pedersen LD, Fischer C, Krstrup P, Bangsbo J: Potassium kinetics in human muscle interstitium during repeated intense exercise in relation to fatigue. *Pflugers Arch* 2004;448:452–456.
- 15 Kofuji P, Newman EA: Potassium buffering in the central nervous system. *Neuroscience* 2004;129:1045–1056.
- 16 Perez FR, Venegas F, Gonzalez M, Andres S, Vallejos C, Riquelme G, Sierralta J, Michea L: Endothelial epithelial sodium channel inhibition activates endothelial nitric oxide synthase via phosphoinositide 3-kinase/Akt in small-diameter mesenteric arteries. *Hypertension* 2009;53:1000–1007.
- 17 Fujiwara N, Osanai T, Kamada T, Katoh T, Takahashi K, Okumura K: Study on the relationship between plasma nitrite and nitrate level and salt sensitivity in human hypertension: modulation of nitric oxide synthesis by salt intake. *Circulation* 2000;101:856–861.
- 18 Li J, White J, Guo L, Zhao X, Wang J, Smart EJ, Li XA: Salt inactivates endothelial nitric oxide synthase in endothelial cells. *J Nutr* 2009;139:447–451.
- 19 Fels J, Callies C, Kusche-Vihrog K, Oberleithner H: Nitric oxide release follows endothelial nanomechanics and not vice versa. *Pflugers Arch* 2010;460:915–923.
- 20 Bussemaker E, Hillebrand U, Hausberg M, Pavenstadt H, Oberleithner H: Pathogenesis of hypertension: interactions among sodium, potassium, and aldosterone. *Am J Kidney Dis* 2010;55:1111–1120.
- 21 Fels J, Oberleithner H, Kusche-Vihrog K: Ménage à trois: aldosterone, sodium and nitric oxide in vascular endothelium. *Biochim Biophys Acta* 2010;1802:1193–1202.
- 22 Oberleithner H, Kusche-Vihrog K, Schillers H: Endothelial cells as vascular salt sensors. *Kidney Int* 2010;77:490–494.