Ideas, Conjectures and Refutations

Vascular endothelium: a vulnerable transit zone for merciless sodium

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ABSTRACT

In humans, when plasma sodium concentration rises slightly beyond 140 mM, vascular endothelium sharply stiffens and nitric oxide release declines. In search of a vascular sodium sensor, the endothelial glycocalyx was identified as being a negatively charged biopolymer capable of selectively buffering sodium ions. Sodium excess damages the glycocalyx and renders vascular endothelium increasingly permeable for sodium. In the long term, sodium accumulates in the interstitium and gradually damages the organism. It was discovered that circulating red blood cells (RBC) report surface properties of the vascular endothelium. To some extent, the RBC glycocalyx mirrors the endothelial glycocalyx: A poor (charge-deprived) endothelial glycocalyx causes a poor RBC glycocalyx and vice versa. This observation led to the assumption that the current state of an individual’s vascular endothelium in terms of electrical surface charges and sodium-buffering capabilities could be read simply from a blood sample. Recently, a so-called salt blood test was introduced that quantifies the RBC sodium buffer capacity and thus characterizes the endothelial function. The arguments are outlined in this article spanning a bridge from cellular nano-mechanics to clinical application.

Keywords: blood vessel, glycocalyx, hypertension, salt sensitivity, sodium channels

PROLOGUE

For millions of years, daily salt (sodium chloride) intake in man was about 1 g. Then recently, about 10 000 years ago, salt intake increased by about 10-fold because of the practice of using salt as a food preservative [1, 2]. It allowed former nomads to settle, grow grain and preserve food stuff over long periods. Over the last few millennia, humans got used to the taste of salt and enjoyed the benefits of non-perishable food [3]. However, the human genome could not adapt so quickly. Genetically, humans are well equipped with mechanisms that retain even tiny amounts of salt, a prerequisite for survival in those times when salt was scarce and intake was low.

Returning to the present, at least 30% of the world’s population develops arterial hypertension when exposed to a high-salt diet [4]. Salt sensitivity, i.e. the development of hypertension in response to salt, differs among people. The reasons for the different salt sensitivities are largely unknown. It could be related to the intrinsic ability of the kidneys to excrete sodium [5], to the limited storage capacities of skin and muscle [6] and/or to a variable sodium buffer capacity of the vasculature [7]. The fact that the vascular system is also a potential target for aldosterone led to a paradigm shift in so far as the attention was no longer directed solely to the kidney, but also to the vascular system [8–13].

ENDOTHELIUM SENSES SODIUM

The luminal wall of all blood vessels is lined by a thin monolayer of cells, the vascular endothelium. This endothelial cell (EC) layer serves a number of important functions. It is (i) a selective mechanical barrier between blood and tissue [14, 15], (ii) a biochemically highly active cell layer in the control of blood clotting processes [16] and (iii) a key regulator of vascular smooth muscle tone [17]. Sodium and aldosterone act synergistically on vascular endothelium. At the cellular level, small changes in plasma sodium concentration can have a large impact on endothelial function as long as intracellular aldosterone receptors are functional [18]. Even a 2% increase in plasma sodium concentration beyond 140 mM mechanically stiffens endothelial cells by about 20% leading to cellular
increase in blood pressure [30]. For obvious reasons, this more
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ing sympathetic nerve activity, but it also acts on blood vessels.
glands [29]. Endogenous ouabain acts in the brain by increas-
endogenous ouabain in the hypothalamus and suprarenal
concentration in the cerebrospinal
[26, 28]. Recently, it was postulated that a high sodium con-
involved in the sodium-triggered increase of blood pressure
Finally, there is experimental evidence that the brain may be
ated by concomitant changes in blood pressure, are known to
occur in humans during acute or chronic salt loading [26, 27].

There is accumulating evidence that the eGC plays a promi-
nent role as a buffer barrier for sodium. In a previous in vitro
study, the eGC sodium store was calculated and extrapolated
to in vivo conditions [7]. At a low-sodium condition, mi-
micked in vitro by maintaining endothelium in culture over
days in a low sodium medium (135 mM), the total eGC

Dysfunction (Figure 1). A major component of this high
sodium sensitivity is based on a selective sodium channel in
the endothelial plasma membrane [19, 20] which is similar to
the epithelial sodium channel cloned from renal tissue [21].
This channel allows sodium to enter the endothelial cells [22]
and, by yet unknown mechanisms, turns off endothelial nitric
oxide synthase activity [23].

The question whether in vitro experiments translate into the
in vivo setting is not easy to answer, since changes in plasma sodium are usually accompanied by changes in osmol-
ality which may mask any direct action of sodium. However, a
study properly corrected for any changes in osmolality shows
that there is indeed a marked alteration in blood pressure
when plasma sodium is manipulated [24]. Similarly, blood
pressure in dialysis patients is known to decrease when sodium concentration in the dialysate is lowered [25]. Further-
more, small but significant changes in plasma sodium, paral-
leled by concomitant changes in blood pressure, are known to
occur in humans during acute or chronic salt loading [26, 27].
Finally, there is experimental evidence that the brain may be
involved in the sodium-triggered increase of blood pressure
[26, 28]. Recently, it was postulated that a high sodium con-
centration in the cerebrospinal fluid triggers the secretion of
endogenous ouabain in the hypothalamus and suprarenal
glands [29]. Endogenous ouabain acts in the brain by increas-
ing sympathetic nerve activity, but it also acts on blood vessels.
Both endogenous ouabain actions lead to vasoconstriction and
increase in blood pressure [30]. For obvious reasons, this more
complex mechanism involving different organs cannot be used to explain the in vitro effect of sodium.

**ENDOTHELIAL GLYCOCALYX BUFFERS SODIUM**

The luminal surface of blood vessels is covered by a gel-like
anionic biopolymer, the endothelial glycocalyx (eGC) [31–33].
This negatively-charged surface layer is hundreds of nano-
metre thick, prevents unspecific adhesion of circulating
blood cells, slows down blood flow in the capillary system
[34] and selectively controls endothelial sodium permeability
[22, 35].

The eGC controls flow- and pressure-induced mechano-
transduction of the endothelium [33, 36] and possibly plays a
crucial role in the pathogenesis of inflammation [37, 38].
Chemically, the eGC is an anionic biopolymer with specific
ion binding properties [39]. Negatively charged heparan sul-
phate residues offer binding sites for sodium. Probably,
calcium ions control eGC conformation in a way that sodium
ions are preferentially adsorbed [39, 40]. It has been shown
that sodium excess, mimicked in ex vivo experiments by a 10% increase of extracellular sodium, leads to the deterioration
of the eGC [7]. That aldosterone is a mediator of this process is
probably due to the insertion of sodium channels into the
plasma membrane [19, 20, 41]. Deterioration of the eGC
allows sodium to enter readily into the cells via the sodium
channels where it disturbs cellular function. The relationship
between ‘thickness’ and ‘stiffness’ of the eGC is illustrated in
Figure 2. A stiff eGC, as observed in sodium excess (i.e.
elevation of extracellular sodium for days), is a ‘collapsed’ eGC
which lacks heparan sulphates and thus has reduced sodium
buffer capacity [7].

There is accumulating evidence that the eGC plays a promi-
nent role as a buffer barrier for sodium. In a previous in vitro
study, the eGC sodium store was calculated and extrapolated
to in vivo conditions [7]. At a low-sodium condition, mi-
micked in vitro by maintaining endothelium in culture over
days in a low sodium medium (135 mM), the total eGC

![Figure 1: Relationship between extracellular sodium concentration and endothelial cell stiffness. The black dotted lines indicate the respective slopes. The reference value for the black curve is 136 mM sodium. The red dotted line connects data points obtained in human plasma of one particular sample which was adjusted to two different sodium concentrations. The reference value is a sodium concentration of 137 mM measured initially in the volunteer’s plasma. Isosmolality was achieved by appropriate additions of mannitol. The inset (lower right) describes the indentation technique using an atomic force microscope (modified after [18]).](image1)

![Figure 2: Correlation between endothelial glycocalyx stiffness and glycocalyx thickness. Experiments were performed in arteries ex vivo exposed for 1 week to low (135 mM) sodium, high (150 mM) sodium and high-sodium plus spironolactone (100 nM). Aldosterone (0.1 nM) was present in all media (modified after [7]).](image2)
sodium store of the human vasculature is supposed to be about 0.7 g sodium. At a high-sodium condition (150 mM), the eGC sodium store is impaired and found reduced by about two thirds.

SODIUM CYCLING BETWEEN BLOOD AND INTERSTITIUM

How fast is sodium eliminated by the kidneys after ingestion of a salty meal, and what is the role of the vascular endothelium in this context? Figure 3 displays a physiologist’s view. It is assumed that an intact glycocalyx has a sodium buffering capacity of about 7 g (see above) which is about the amount of sodium found in a salty snack. Two extreme examples illustrate what may happen after ingestion of this amount of salt:

The first example is an individual with low sodium sensitivity, i.e. with a high sodium buffer capacity of the eGC. This individual has an intact glycocalyx and a low number of sodium channels in the endothelium. When the ingested sodium arrives in the circulation, it will tend to raise sodium in the blood where it will be retained for a short period. This is due to a sufficient buffering capacity of the eGC (first barrier) and the low sodium permeability of the endothelial cells (second barrier). Thus, sodium is rapidly eliminated by the kidneys.

The second example is an individual with a high sodium sensitivity, i.e. with a more or less collapsed eGC and a high number of sodium channels in the endothelium. In contrast to the first example, in this case the same amount of ingested sodium arriving in the circulation will more easily exit the vascular system due to the insufficient buffer capacity of the deteriorated eGC and the high sodium permeability of the endothelial cells. Thus, in these circumstances, sodium is readily distributed in the large interstitial compartment of the whole body where it is transiently bound in an osmotically inactive form to the negative charges of the extracellular matrix [42]. Thus, the ingested sodium will not be immediately eliminated via the kidney, but will rather gradually diffuse from the interstitium back into the circulation so that its excretion by the kidneys is delayed.

These two examples represent two extreme conditions. ‘Real life’ conditions are expected to be somewhere ‘in between’. In fact, the ‘transcellular’ sodium permeabilities are beyond doubt much lower than the sodium permeabilities of the ‘paracellular’ pathways. However, despite a rather marginal contribution of a transcellular sodium leak compared with the overall trans-endothelial sodium leak of the endothelial barrier, this scenario may become increasingly important over time. Probably, after months or even years, this mechanism may lead to clinical symptoms.

CROSS-TALK BETWEEN VASCULAR ENDOTHELIUM AND RED BLOOD CELLS

One of the major functions of the glycocalyx is to prevent red blood cells (RBC) from getting attached to the endothelial surface. The negatively charged heparan sulphates, located right at the interface between ‘moving blood’ and ‘resting vessel wall’, are most likely key elements signalling changes in shear stress, blood flow and composition of the blood to the underlying endothelial monolayer [31, 43, 44]. This explains the severe medical problems that can occur if the glycocalyx is damaged. Among them are vascular permeability changes.

FIGURE 3: Schematic illustration how sodium is handled in an organism of low (left) and high (right) sodium sensitivity (modified after [35]).
Correlation between red blood cell (RBC) glycocalyx and endothelial glycocalyx. The strong correlation indicates direct cross-talk between blood and vessel surface (modified after [55]).

during inflammatory processes [45], endothelial dysfunction in chronic kidney failure [46] and atherothrombosis [47].

Similar to the vascular endothelium, erythrocyte membranes are covered by a glycocalyx which, besides sialic acid [48], also contains negatively charged heparan sulphates [49–51]. Thus, the poor adhesiveness of RBC to EC is explained by electrostatic repulsive forces caused by the zeta-potential as described for RBC many years ago [52]. Over the 120 days life span of an individual RBC, the erythrocyte membrane gradually ages as indicated by a decrease in glycocalyx thickness [53, 54]. This is not unexpected because RBC lack nuclei and thus cannot compensate any losses of surface molecules in the long run. In addition, RBC mechanical stress is particularly high during turbulent blood flow at bifurcations of large arteries and during slipping through the narrow capillaries. A recent study indicates that RBC and EC on contact may influence each other [55]. EC with a charge-depleted surface lead to RBC with similar properties and vice versa (Figure 4). The reason for this adaptation process is most likely a mechanical interaction between the ‘floating’ RBC and the ‘stationary’ EC [56, 57].

The ‘cross-talk’ between RBC and EC could have relevance for the progression of cardiovascular diseases and in medical diagnostics. Extrapolating the in vitro results to in vivo conditions, one can assume that a vicious cycle could start when either RBC or EC surface layers are damaged for reason. There is supporting evidence that a poor vascular function is often found in parallel with poor RBC conditions. Uremic patients suffer from endothelial dysfunction, loss of the glycocalyx barrier [46] and anaemia. Although the latter is explained by a lack of renal erythropoietin, RBC membranes were found to be abnormal and RBC life span found to be reduced [58, 59]. A similar parallelism (i.e. endothelial dysfunction combined with RBC abnormalities) can be observed in sepsis [60], genetic hypertension [61], sickle cell anaemia [62] and malaria [49]. These observations support the hypothesis that the physical interaction between erythrocyte and endothelial surfaces becomes relevant in pathological states, i.e. a damaged endothelial surface damages the erythrocyte surface and vice versa.

**The Salt Blood Test**

As described above, RBC ‘mirror’ EC properties. In more detail: when heparan sulphates are enzymatically removed from the endothelial glycocalyx, then RBC will lose these important glyocalyx components as a result of physical interaction. This observation, namely that the surface of RBC obviously ‘memorizes’ the surface of the endothelium, led to the assumption that it should be sufficient to establish a functional test for assessing the properties of the RBC, thus providing insight into the properties of the respective endothelium. This concept underlies the so-called salt blood test (SBT).

The SBT is based on the sodium-dependent RBC zeta potential [52, 63]. Its magnitude is directly related to the negative surface charges. Apart from sialic acids [64], heparan sulphates are supposed to also contribute to the RBC zeta potential. Extrapolated from observation in vascular endothelium, sodium is preferentially bound to these negative charges [40]. It is supposed to be a high-capacity low-affinity binding. Obviously, at low-to-normal plasma sodium concentrations (values below 140 mM), the eGC sodium buffer capacity is close to saturation, but not yet fully saturated. However, at normal-to-high plasma sodium concentrations (beyond 140 mM), the eGC binding sites for sodium ions are fully saturated.

The erythrocyte sedimentation rate (ESR) is used as a quantitative measure for the sodium-dependent zeta potential [52, 63]. In contrast to the standard ESR commonly used as a non-specific test to detect inflammatory or tumorous processes in clinical medicine [65], the SBT is performed in plasma-free electrolyte solution. Besides various concentrations of NaCl, only sucrose (for osmolality reasons) and dextran (crucial for triggering aggregation) are present in the Na+ cocktails. Therefore, the only key variable in the various RBC suspensions is Na+. This makes the test specific for sodium.

Two different Na+ cocktails are sufficient for performing the SBT. The 125 mM Na+ cocktail sets the reference value of the ESR. The 150 mM Na+ cocktail sets the upper limit. The dimensionless erythrocyte salt sensitivity (ESS: Na150/Na125) is a measure for the salt sensitivity of an individual. It would have the value ‘1’ when the increase of Na+ from 125 to 150 mM would not change the zeta potential at all. This constellation would indicate sodium resistance, a theoretical value because this case will never occur. A value above ‘1’ indicates sodium sensitivity, i.e. the larger this value is, the more sensitive to sodium is the individual’s vasculature. Two realistic examples are given in Figure 5.

In a cohort of 61 healthy young individuals, two ESS peaks were detected (Figure 6). This could be due to a genetic predisposition [66], but also due to distinct differences in life style [67]. Possibly, the left peak includes individuals with a ‘low’ sensitivity to sodium, while the right peak includes individuals with ‘marked’ sensitivity to sodium. Gender differences cannot explain these two peaks [68].

The SBT will gain medical relevance only if the underlying concept can be proven, i.e. that the sodium buffer capacity of the endothelial glycocalyx (related to the inverse of ESS) can
indeed be evaluated, at least \textit{in vitro}. Recent experiments using the vasoprotective compound WS1442 give some first indications. This crataegus extract WS1442 is known to improve endothelial function \cite{69} and to specifically interact with the endothelial glycocalyx. At least \textit{in vitro}, WS1442 has been shown to enfold the glycocalyx lowering endothelial sodium permeability \cite{70}. Indeed, ESS significantly decreased by about 20\% when the endothelium was pretreated for several days with this compound. Although this is only a very first step in the ‘proof of concept’, it opens some interesting perspectives. Sodium sensitivity at the level of the vascular system is probably not a ‘constant’ parameter of an individual, but is rather a variable that can be influenced negatively or positively. This view could make the SBT a potentially valuable test system to control medical measures undertaken to improve vascular function.

\textbf{FIGURE 5:} Erythrocyte sodium sensitivity measurements (ESS) indicating ‘weak’ erythrocyte Na\(^+\) sensitivity (blood #1) and ‘strong’ erythrocyte Na\(^+\) sensitivity (blood #2) (modified after \cite{68}).

\textbf{FIGURE 6:} Frequency distribution of the erythrocyte sodium sensitivity (ESS) in a cohort of 61 study participants (modified after \cite{68}).

\textbf{SALT PROVOCATION TEST VERSUS SALT BLOOD TEST}

The so-called salt provocation test (SPT) had been introduced in the past \cite{71}. Since both the SPT and the SBT were developed in our laboratory over the past years, I wish to directly compare them, pointing out their specific strengths and weaknesses. Both tests share a common goal, namely quantifying the individual sodium sensitivity in humans with the clear focus on the functional state of the blood vessels. Both tests share a simple design that does not require sophisticated equipment and that causes only minimal stress to the individual.

The SPT is based on the concept ‘the more sodium-permeable the endothelium is, the more sodium-sensitive is the individual’. This hypothesis is tested by two oral salt loads (5 g), in the absence and presence of a sodium channel blocker (100 mg amiloride). The read-out is the diastolic blood pressure measured over 1 h in response to the two treatments \cite{71}. The SPT gives ‘functional’ insight into the quality of the endothelial surface. The weaknesses of this test are (i) the inevitable scatter of blood pressure measurements, (ii) the application of two oral salt loads and (iii) the oral application of a single amiloride dose. The strengths of the SPT are that (i) it reflects the ‘functional’ state of the blood vessels and (ii) a standard blood pressure monitoring device is the only instrumental prerequisite to perform this test.

The SBT is based on the concept ‘the higher the sodium-dependent RBC sedimentation rate, the more sodium-sensitive is the individual’. This test needs a small blood sample that is used to quantify erythrocyte sedimentation in two different sodium cocktails (details in \cite{68}). Similar to the SPT, the SBT provides insight into the quality of the endothelial surface while using an \textit{in vitro} approach. The weaknesses of this approach are that (i) blood has to be collected by venipuncture (though the test can be probably miniaturized to a 200-µL blood sample taken from the fingertip) and (ii) a small

\textbf{FIGURE 7:} Significant correlation between data of the salt provocation test (SPT) and the salt blood test (SBT) obtained in the same individuals. The SPT values are ‘Δ diastolic pressure’ values (mmHg) \cite{71}. The SBT values are dimensionless ESS values.
CONCLUSIONS

At the end I would like to return to the title of this article ‘Vascular endothelium: a vulnerable transit zone for merciless sodium’.

The word ‘vulnerable’ should indicate that excessive amounts of sodium harm the delicate surface layer of the endothelium.

The term ‘transit zone’ should indicate that incoming sodium finds a temporary shelter within the endothelial glyocalyx.

The term ‘merciless sodium’ refers to the intrinsic property of this cation, namely that large quantities finally damage the whole organism.

ACKNOWLEDGEMENTS

This article is dedicated to Prof. Hugh de Wardener in memory of his enthusiastic support of our research over many years. Work was supported by the Deutsche Forschungsgemeinschaft (Koselleck-grant OB 63/18). The article is based on six recent reports from the author’s laboratory [7, 35, 55, 68, 71, 72].

CONFLICT OF INTEREST STATEMENT

None declared.

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Received for publication: 19.9.2013; Accepted in revised form: 25.9.2013