El Endotelio como sensor vascular de sal

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POSSIBLE CLINICAL RELEVANCE OF ENDOTHELIAL CELL STIFFNESS

Force is applied to most tissues in real life, particularly to vascular endothelium. Hemodynamic forces, generated by the beating heart, give rise to shear stress at the endothelial surface. It is inevitable therefore that the apical cell surfaces undergo reversible deformations and that it is this mechanical stimulus that triggers the activity of the endothelial nitric oxide synthase (eNOS) and the release of nitric oxide (NO).

The NO diffusesto the adjacent vascular smooth muscle cells, which relax leadingto vasodilation. This regulatory mechanism distributes the blood in the organism according to the metabolic demands whereas the systemic blood pressure is maintained within physiological limits. The same shear force should cause a stiff (less deformable) cell to release less NO. Therefore, endothelial mechanical stiffness is a key parameter in the control of local blood supply and arterial blood pressure

ATOMIC FORCE MICROSCOPE: A MECHANICAL NANOSENSOR
The ‘tool of choice’ for quantitatively measuring the stiffness (given in N/m) of living adherent endothelial cells is an atomic force microscope (AFM). In principle, the AFM is used as a mechanical tool.

At least two different slopes can be identified depending on the depth of indentation.
The initial rather flat slope (indentation depth: up to a several 100nm) reflects the stiffness of the soft plasma membrane including the cortical cytoskeleton (the cell’s ‘shell’), whereas the late rather steep slope reflects the stiffness of the more rigid cell center.

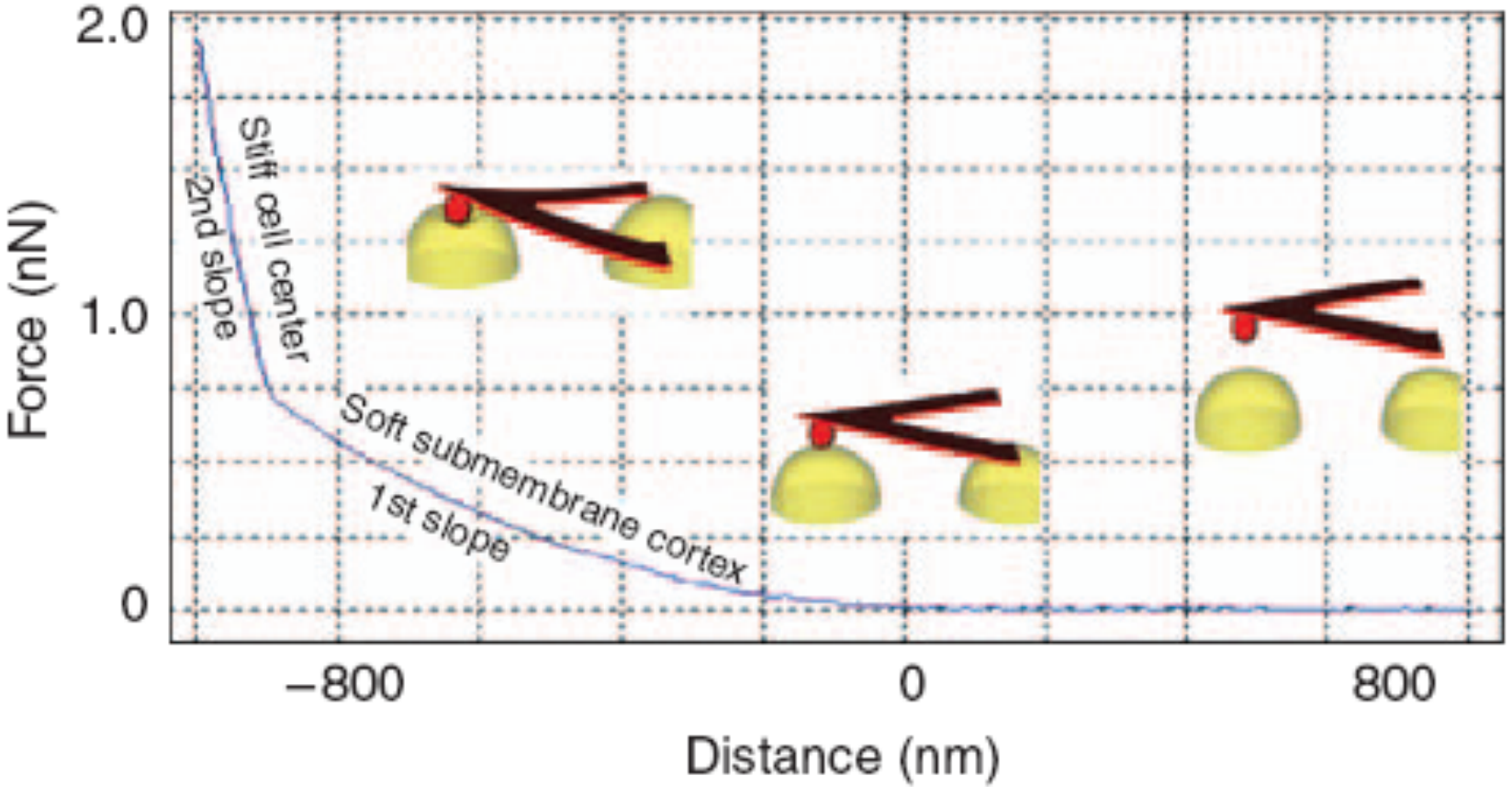


Figure 2 | Indentation technique using atomic force microscopy. Indentation curve with two different slopes (modified from Oberleithner *et al.*[15]).

SODIUM: ‘STIFFENER’ OF VASCULAR ENDOTHELIAL CELLS

For more than three million years primitive man consumed less than 1 g per day of sodium chloride.

About 8000 years ago, with the advent of agriculture and farming, 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.

Although the German engineer Carl von Linde invented the refrigerator more than 130 years ago, which has enabled food to be stored by deep-freezing, modern society has not yet returned to the low levels of sodium consumed during primitive times.

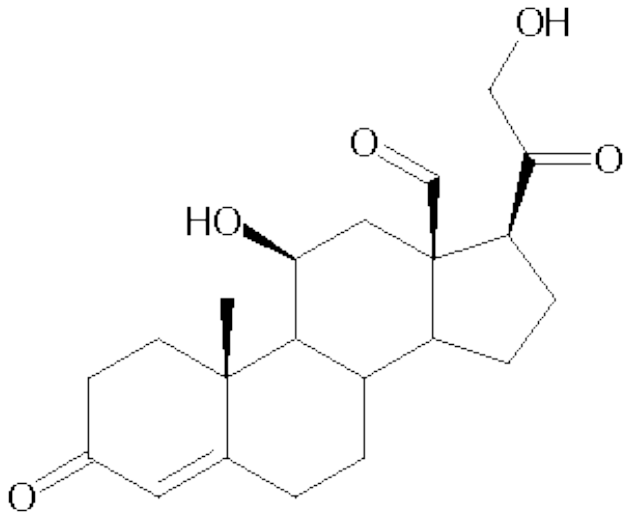
As life expectation is nowadays close to 80 years, the harmful effects of high-salt diets has become increasingly apparent. Hypertension, stroke, coronary heart disease, and renal fibrosis are related to a high sodium intake.

Although the deleterious effects of a high sodium intake are now obvious, the underlying mechanisms how sodium chloride exerts its effect at the organ, tissue, andcellular levels are still unclear.

A high sodium intake causes fibrosis and inflammatory processes in the kidney and heart. When dietary salt intake exceeds renal excretory capacity an increased amount of sodium is stored in the space between cells, bound to extracellular organic material.

Plasma sodium is raised in hypertension (by 3–4 mEq/l) when dietary sodium intake is raised.

It has therefore been postulated that changes in plasma sodium per se may control the blood pressure. Accordingly, the effect of such small changes in sodium concentration on endothelial function has been studied. It was found that when extracellular sodium is raised in this manner endothelial cells stiffen within minutes and that this only occurs in the presence of aldosterone. It is remarkable, however, that endothelial cells appear insensitive to sodium concentrations below 139 mmol/l, above which, however, they are highly sensitive.



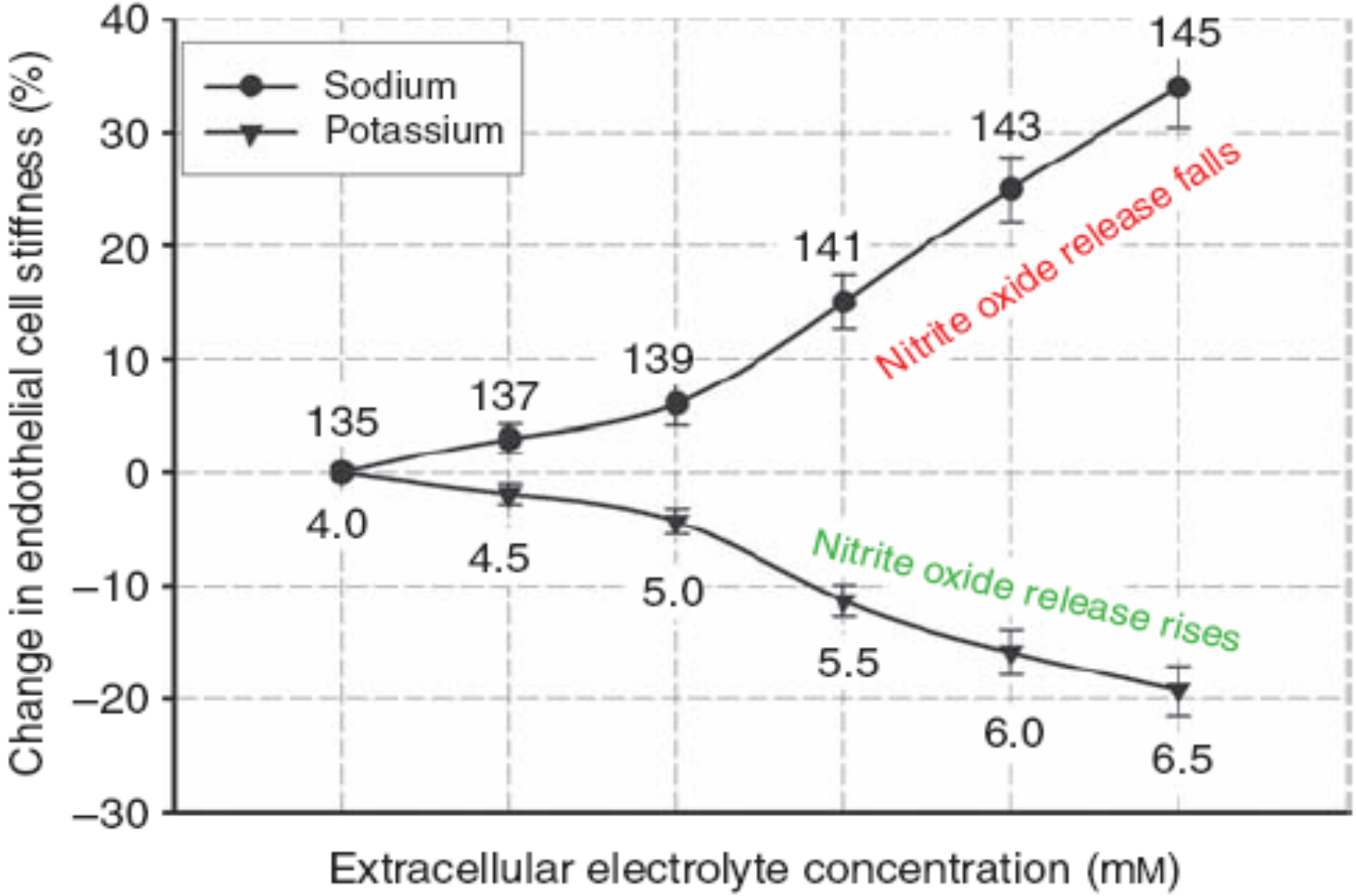
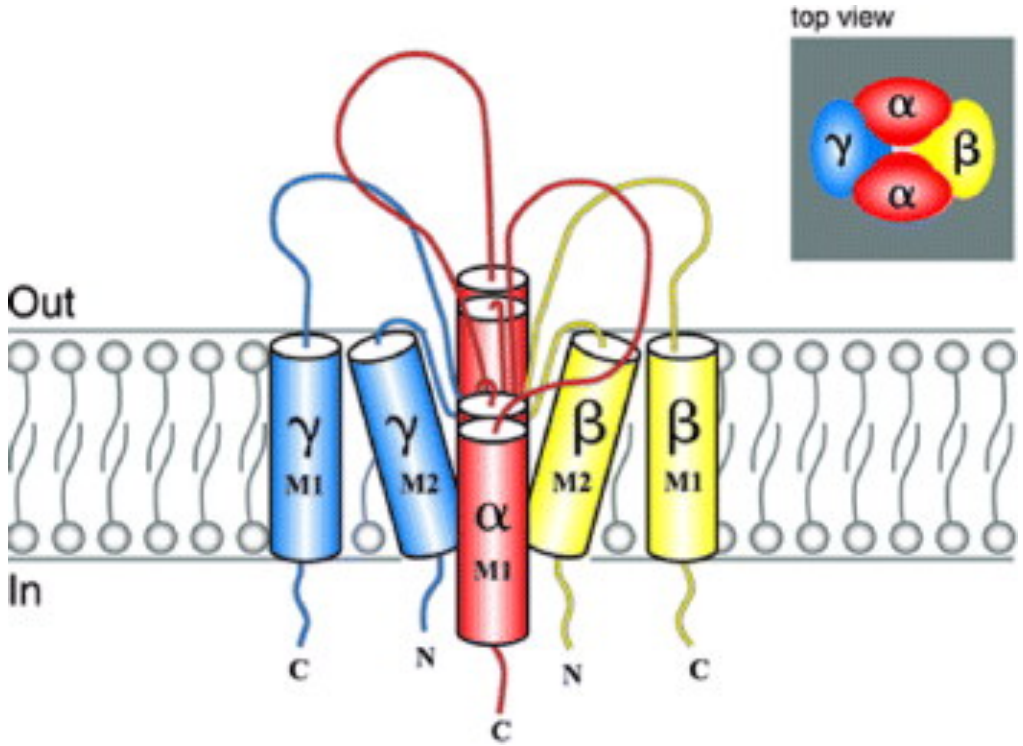


Figure 3 | Relationship between cell stiffness and electrolyte concentrations. Please note that the electrolyte-induced changes in stiffness occur within minutes. Numbers close to the mean values (±s.e.m.) are the respective ion concentrations in the extracellular solution (mmol/l) (modified from Oberleithner *et al.*[9,15]).

Inhibition of the cytosolic mineralocorticoid receptors by spironolactone (or eplerenone) prevents endothelial stiffening as does inhibition of the epithelial sodium channel (ENaC; present in the apical plasma membrane of endothelia) by amiloride.

These in vitro experiments may explain the protective action of these two substances on the cardiovascular system.

The data strongly support the view that the blood vessels and the heart are primary targets for diuretics, independent of any actions they may have on the kidney.



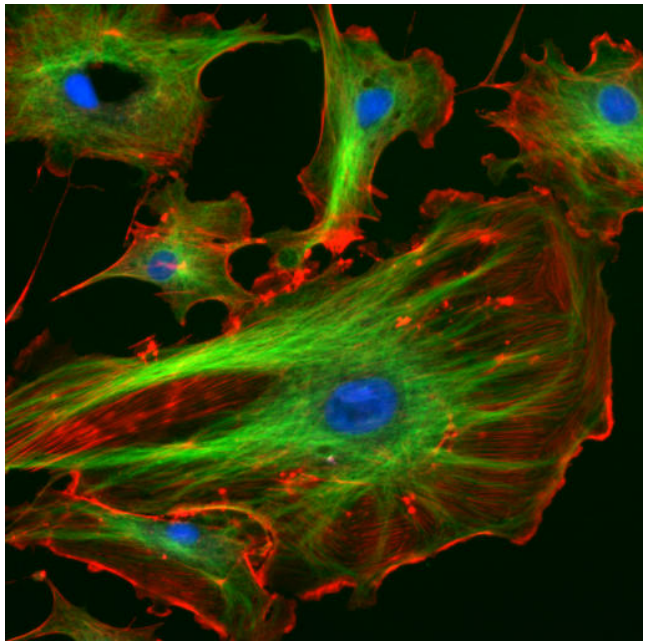
POTASSIUM: SOFTENER OF VASCULAR ENDOTHELIAL CELLS

Besides a genetic predisposition for hypertension, it is the amount of the daily sodium and potassium intake that may lead to onset of elevated blood pressure in the otherwise healthy individual.

In contrast to natural food, processed food products are rich in sodium and poor in potassium.

There is general agreement that a high-potassium, low sodium diet exerts beneficial effects on the cardiovascular system and may even influence emotion such as depression, tension, and vigor.

Potassium deficiency is difficult to detect for 98% of body potassium is intracellular



Extracellular potassium concentrations above 5mEql/l swell and soften endothelial cells. Plasma potassium is often raised in kidney disease but ‘local’ potassium concentrations greater than 5mEq/l, are absolutely normal in muscle during physical exercise and in the brain during increased neuronal activity, while overall electrolyte homeostasis is unaffected.

The extent of potassium-induced cell softening depends on the absence or presence of aldosterone and the concentration of extracellular sodium

ENDOTHELIAL NO RELEASE: OPPOSING ROLES OF SODIUM AND POTASSIUM

Endothelial nitric oxide synthase is located at the caveoli of the apical cell membrane and its expression and/or activity is regulated by various factors. It is stimulated by increases in intracellular Ca (through calmodulin)

Sodium ions control eNOS activity, which is also inhibited by aldosterone,possibly indirectly through EnaC-mediated sodium influx.

Conversely, inhibition of ENaC-mediated sodium influx by amiloride activates eNOS.

An increase in the intake of salt induces the production of asymmetrical dimethyl-L-arginine, a competitive eNOS inhibitor, and increasing extracellular sodium within the physiological range, downregulates eNOS expression and angiogenesis.

There is a negative correlation
between stiffness and eNOS activity,

Extracellular sodium
concentration strongly determines stiffness and eNOS
function, and

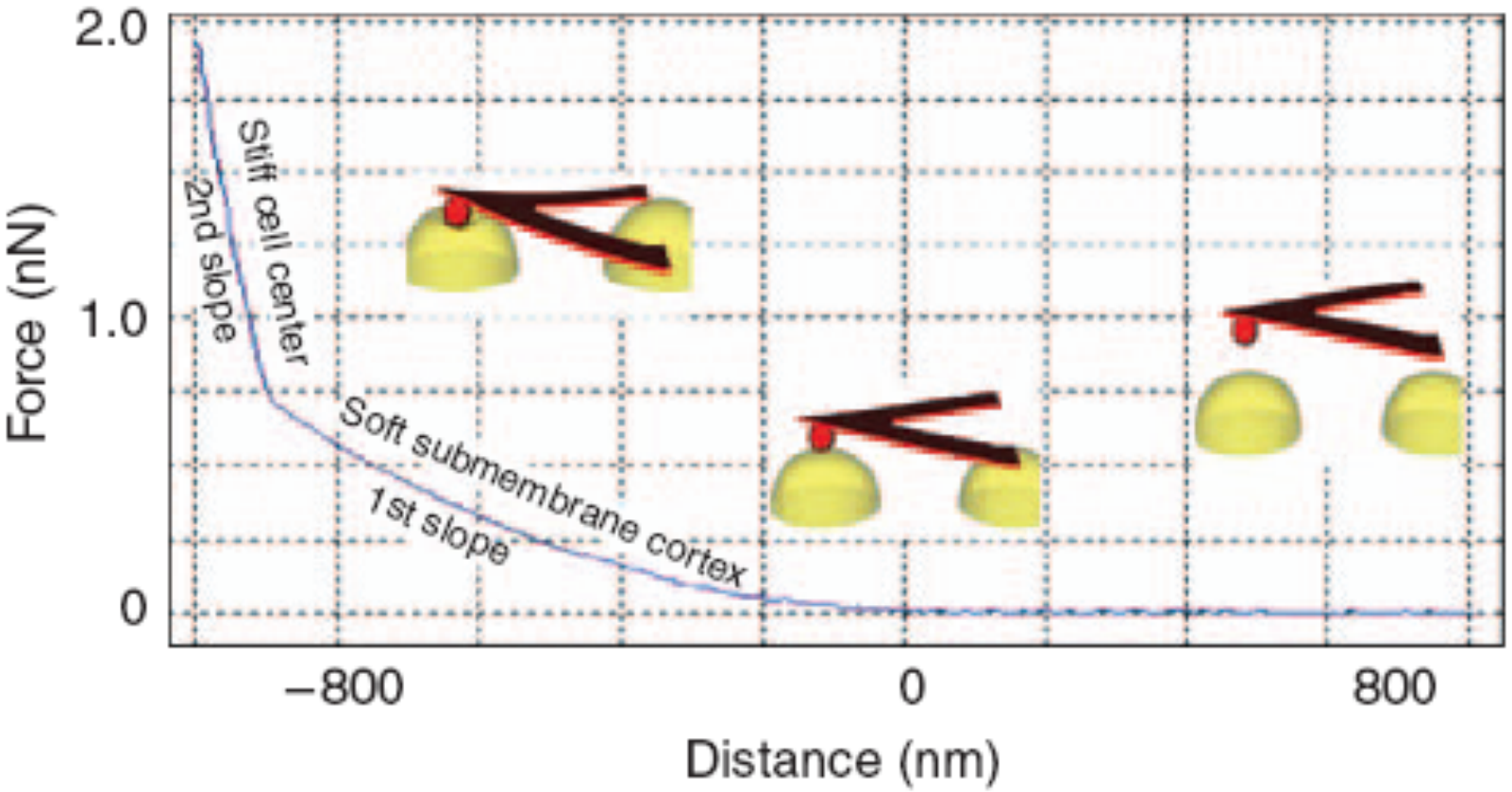
Extracellular potassium concentration
influences eNOS activity and stiffness only at low sodium
concentrations.

‘SOLATION–GELATION’ HYPOTHESIS

Vascular endothelial cells undergo large changes in shape (e.g., during vascular dilation/constriction, particularly in those that occur with each contraction of the heart) and can best adjust to such alterations if the deformability (physical compliance) of the cells is high.

The AFM analysis reveals at least two linear slopes in the indentation curves; the first tends to be flat whereas the second is steeper.

The first flatter slope indicates a low stiffness, which is limited to the submembranous cortex of the cell (the cell’s shell). The nature of the slope indicates clearly that there is a fluidic layer immediately beneath the plasma membrane, which is highly changeable in terms of thickness and viscosity. The cortical cytoskeleton of vascular endothelial cells is highly ‘dynamic’ and the state of polymerization of cortical actin determines the structure and mechanical properties of this layer.



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Monomeric globular actin (G-actin), which can rapidly polymerize into filamentous actin (F-actin), can cause a rapid increase in local viscosity (gelation).

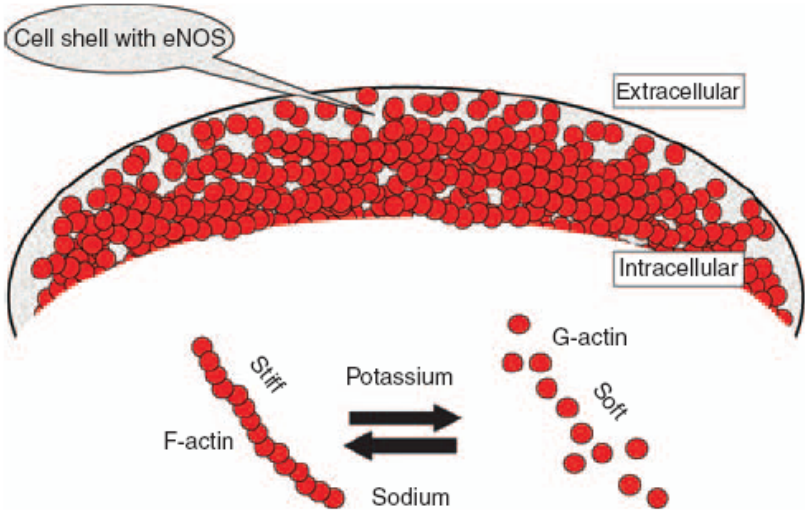


Figure 5 | Hypothesis for how sodium and potassium control the fluidity of the cortical zone ('cell shell') in an endothelial cell.

An increase in extracellular potassium mimicks this response indicating that potassium per se softens the cortical actin cytoskeleton by changing F-actin to G-actin. G-actin is known to colocalize with eNOS and to increase eNOS activity.

This could explain the activation of eNOS by high potassium.

It is possible that in this system sodium is a functional antagonist.
Sodium influx, mediated by aldosterone-activated ENaC, stiffens the cytoskeleton by increasing the viscosity of the submembranous layer.
It is hypothesized that when sodium is in the high physiological range, filamentous actin dominates over monomeric actin.

This would explain the sodium-induced increase in cell stiffness. When potassium is elevated, actin filaments disaggregate into actin monomers and endothelial cells soften.

Both F-actin and G-actin are negatively charged molecules and their interaction with Na and K will finally depend on the local concentrations and specific affinities of the respective ions directly beneath the plasma membrane, most likely at the caveolae.

As this cytosolic submembranous zone (cell shell) is only a few hundred nanometers thick, it is implied that about 90% of the cell's body remains uninvolved.

Sodium has a greater affinity to protein surfaces than potassium.It is assumed that when sodium enters the cell (e.g., through ENaC activation) it binds with high affinity to actin displacing potassium from the carboxylate groups within the amino-acid side chains.

Thus, increasing theconcentration of sodium, which has a higher affinity to actin as compared to potassium, effectively modulates its protein–protein interaction strength.

Such small changes in sodium or potassium in the submembranous zone should control the state of actin polymerization and thus the cell stiffness and functionality

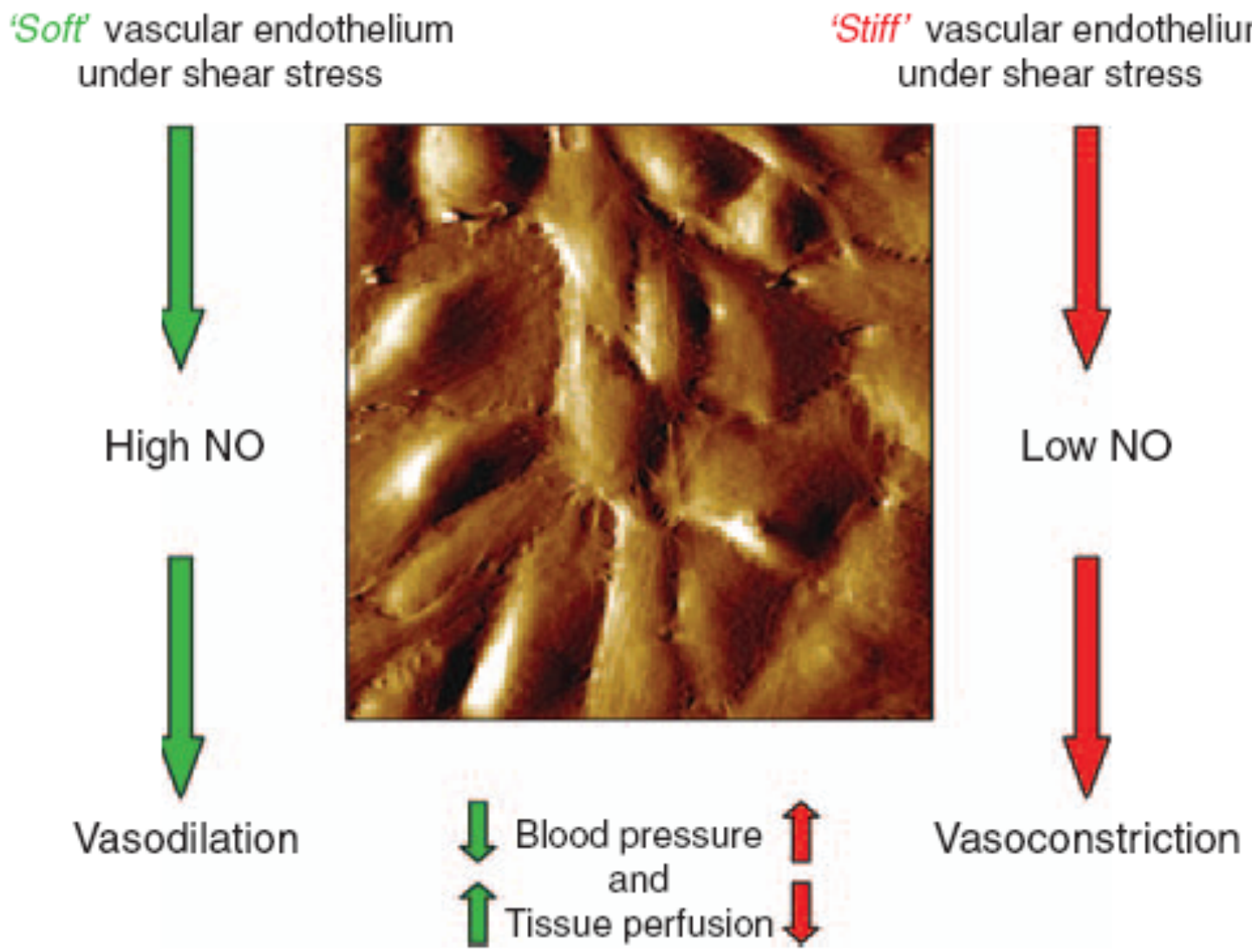
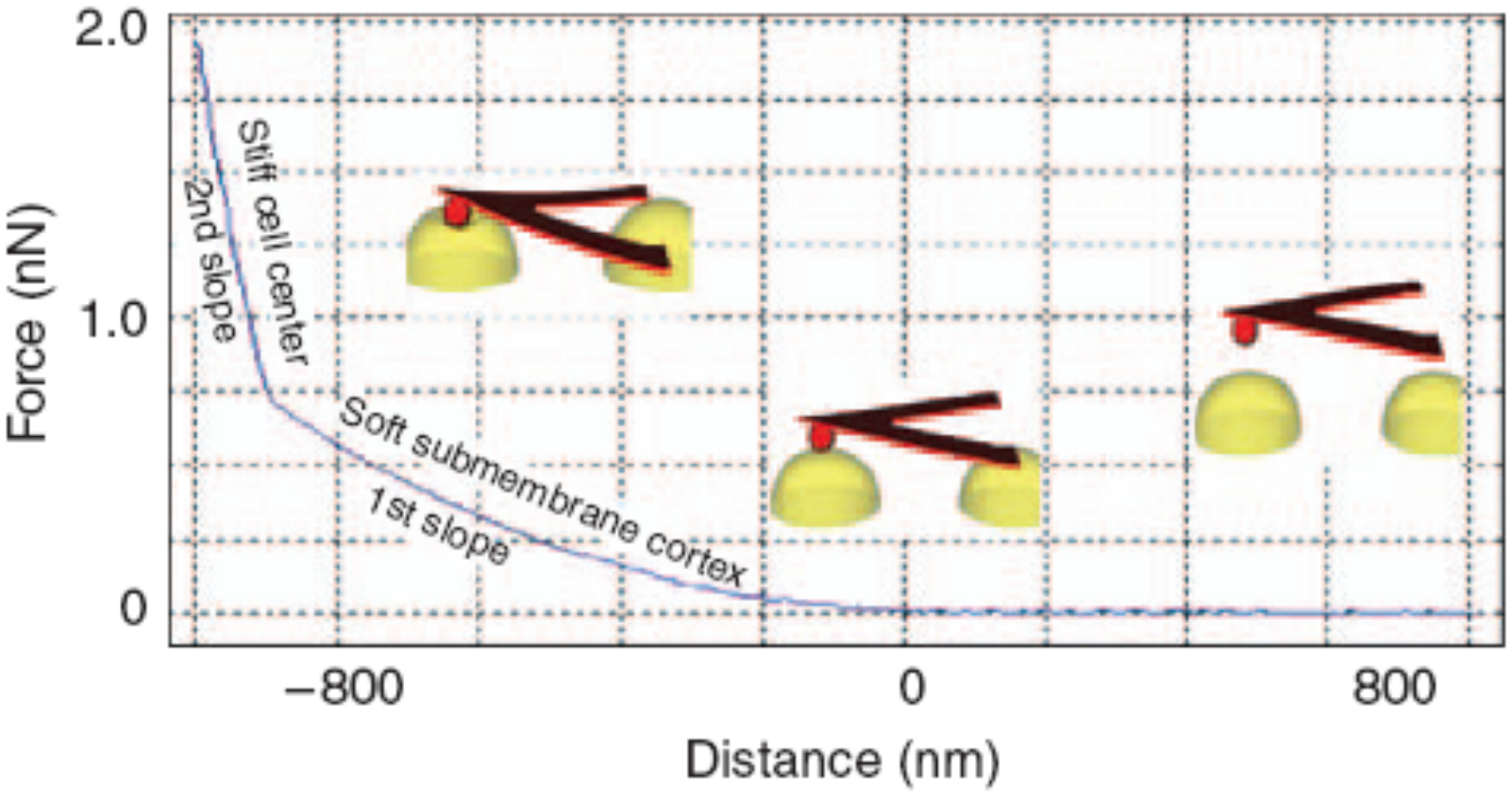


Figure 1 | Concept of how the mechanical stiffness of endothelial cells participates in the regulation of blood pressure.



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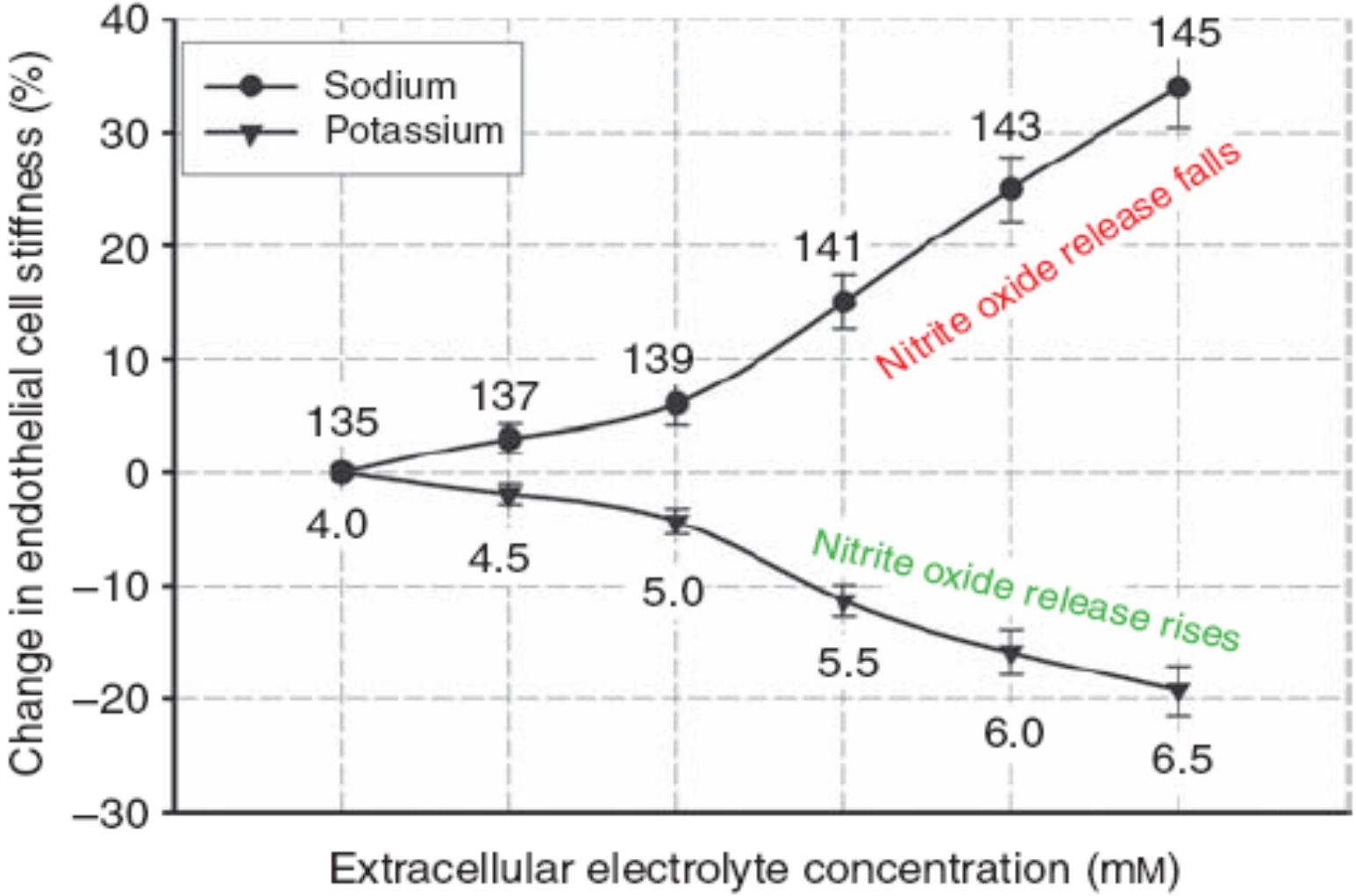

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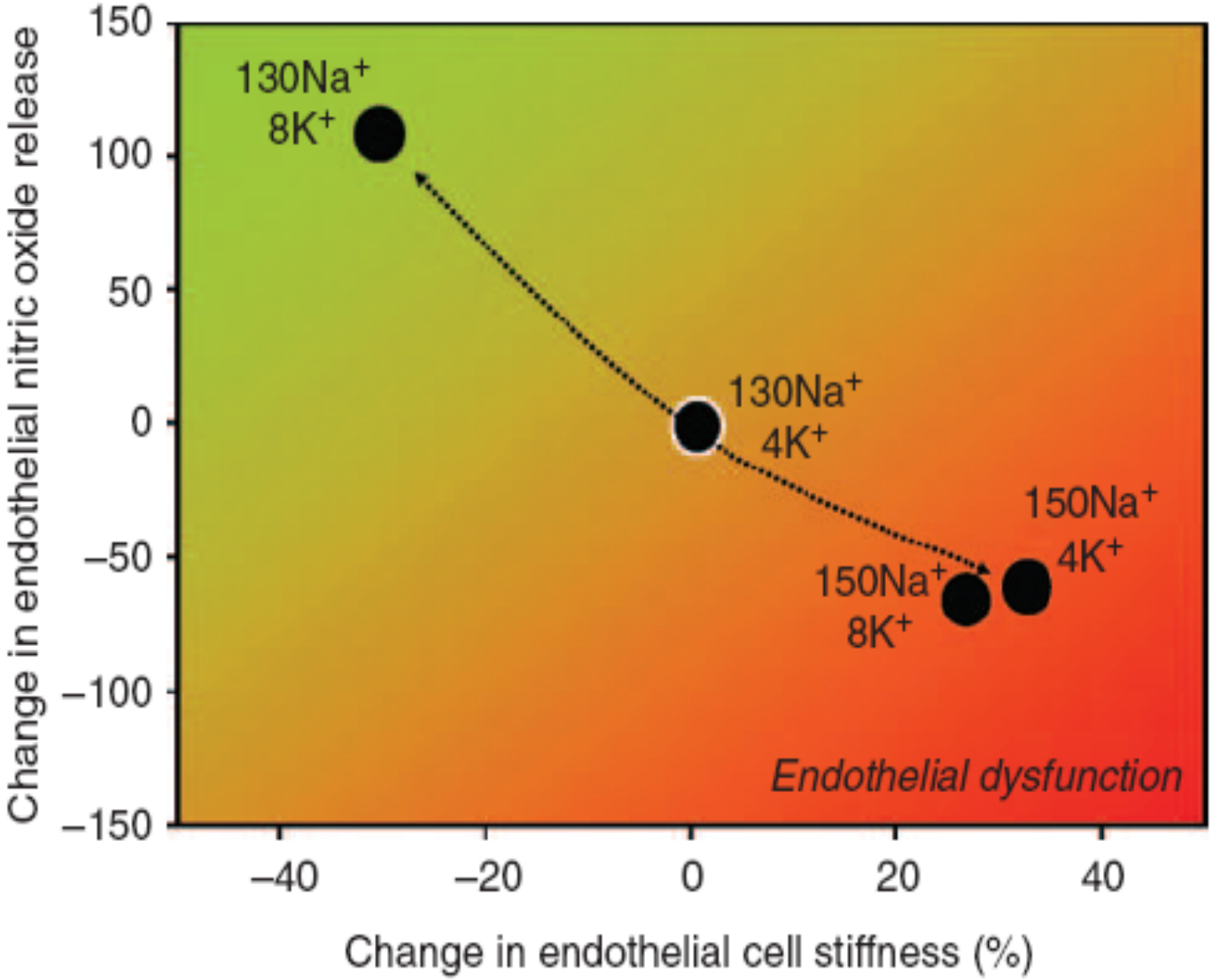
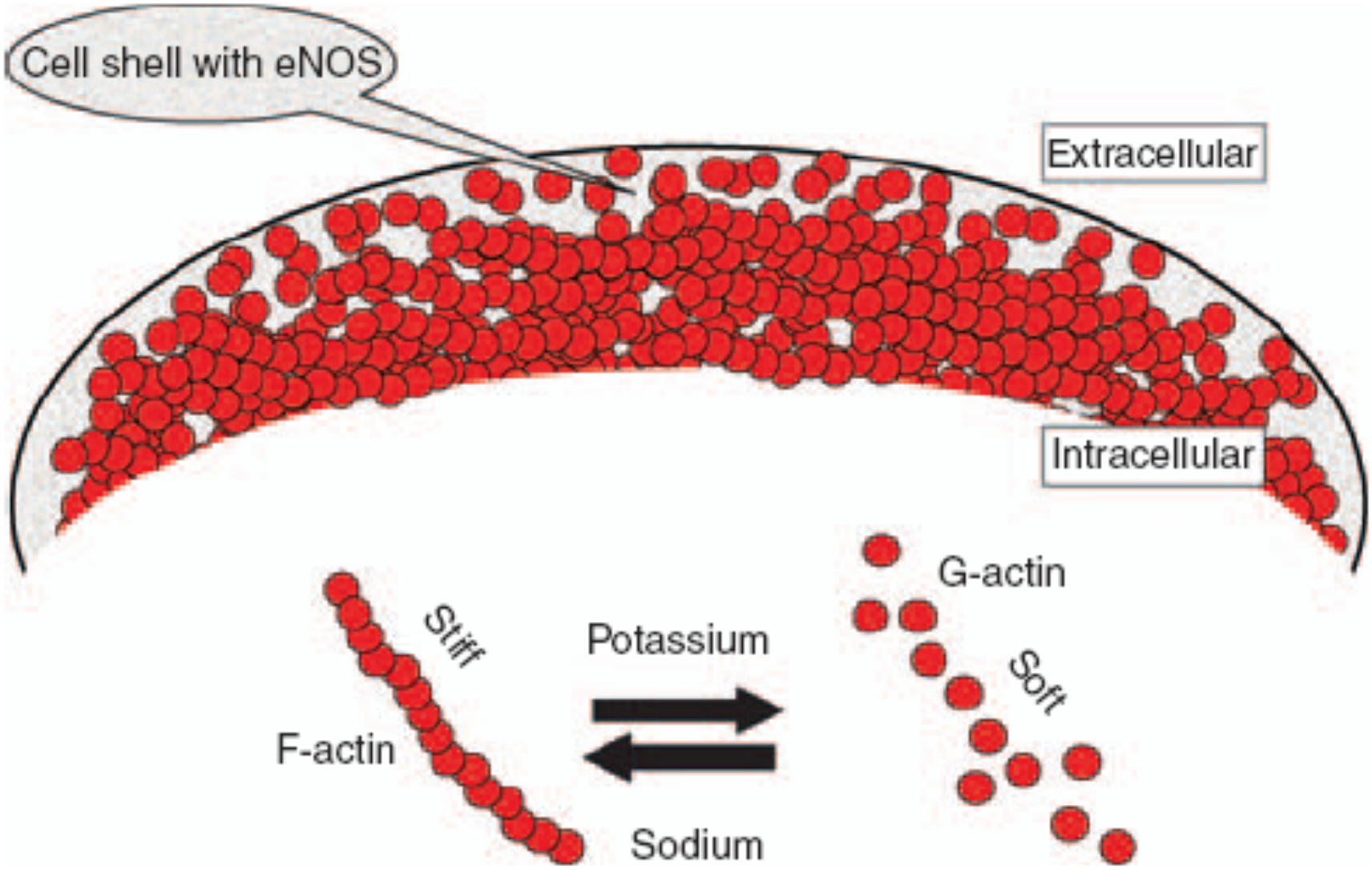


Figure 4 | Negative correlation between cell stiffness and nitric oxide (NO) release. NO release was derived from the nitrite concentrations measured in the supernatant culture media. Data were taken from Oberleithner *et al.*[9,15]



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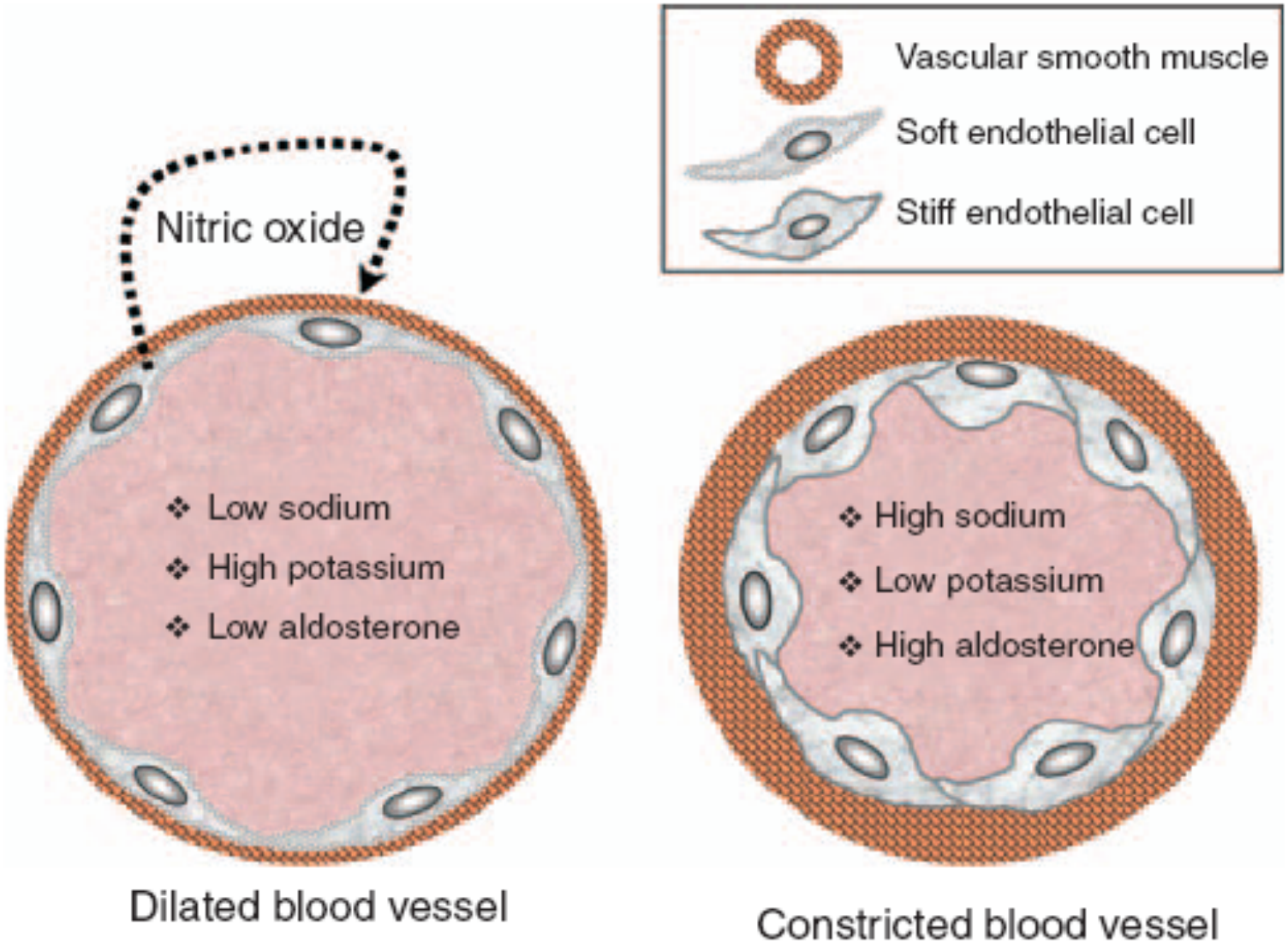

Figure 6 | Concept of how sodium, potassium, and aldosterone contribute to the regulation of blood vessel tone.

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