Potassium softens vascular endothelium and increases nitric oxide release

H. Oberleithner[a,1], C. Callies[a], K. Kusche-Vihrog[a], H. Schillers[a], V. Shahin[a], C. Riethmüller[a], G. A. MacGregor[b], and H. E. de Wardener[b]

[a]Institute of Physiology II, University of Münster, D-48149 Münster, Germany; and [b]Blood Pressure Unit, Department of Cardiac and Vascular Medicine, St. George's University of London, London SW17 ORE, United Kingdom

Communicated by Gerhard Giebisch, Yale University School of Medicine, New Haven, CT, December 21, 2008 (received for review August 20, 2008)

In the presence of aldosterone, plasma sodium in the high physiological range stiffens endothelial cells and reduces the release of nitric oxide. We now demonstrate effects of extracellular potassium on stiffness of individual cultured bovine aortic endothelial cells by using the tip of an atomic force microscope as a mechanical nanosensor. An acute increase of potassium in the physiological range swells and softens the endothelial cell and increases the release of nitric oxide. A high physiological sodium concentration, in the presence of aldosterone, prevents these changes. We propose that the potassium effects are caused by submembranous cortical fluidization because cortical actin depolymerization induced by cytochalasin D mimics the effect of high potassium. In contrast, a low dose of trypsin, known to activate sodium influx through epithelial sodium channels, stiffens the submembranous cell cortex. Obviously, the cortical actin cytoskeleton switches from gelation to solation depending on the ambient sodium and potassium concentrations, whereas the center of the cell is not involved. Such a mechanism would control endothelial deformability and nitric oxide release, and thus influence systemic blood pressure.

aldosterone | blood pressure | cortical actin | epithelial sodium channel | stiffness

A large body of experimental and clinical evidence supports the view of a strong relation between sodium intake and the development of hypertension and cardiovascular disease (1–4). In contrast, potassium intake has been shown to have a beneficial effect on the cardiovascular system (5–9).

Certain steroid hormones, particularly those controlling electrolyte homeostasis in humans (e.g., mineralocorticoids such as aldosterone), have gained increasing attention in relation to cardiovascular disease (10). Endothelial cells are softened by estrogens through activation of a plasma membrane sodium/proton antiporter, yet are insensitive to progesterone and testosterone (11). Similarly, as shown in renal epithelium with scanning ion conductance microscopy (12), aldosterone stimulates endothelial cell volume, growth, and stiffness by activating epithelial sodium channels (ENaCs) (13, 14). In contrast, glucocorticoid hormones do not affect these parameters (15).

Small physiological changes in extracellular sodium concentration directly stiffen vascular endothelium (16). As potassium has a beneficial effect on cardiovascular function (17–20), we have now examined the relation between extracellular potassium and cell stiffness. Here, we show that potassium softens vascular endothelium and increases the release of NO. It is suggested that these changes in stiffness, mediated by potassium and sodium, involve principally a dynamic viscous zone at the periphery of the cell. It is proposed that such a submembrane compartment may rapidly switch between solation (a change toward a fluid-like state) and gelation (a change to a more solid state) depending on ambient sodium and potassium.

Results

Superficial and Deep Stiffness Are Detectable in Living Endothelial Cells. Typical indentation curves of living endothelial cells are not exponential in shape. Because of the improved signal-to-noise ratio with the use of a colloidal atomic force microscopy (AFM) tip instead of a cone (i.e., sharp tip), the indentation curves reveal at least two slopes (Fig. 1). The length of the first linear slope is highly variable from a few nanometers to several hundred nanometers. Deeper indentation leads to the appearance of a second linear slope. In agreement with a previous report (21), we assume that the first slope represents the plasma membrane stiffness and includes a contribution from the submembrane cortical cytoskeleton, whereas the second slope, which is approximately three times larger than the first slope, represents the stiffness of the inner bulk of the cell. Acute changes in sodium or potassium affect only the first slope that is produced by the cell's shallow outer shell and leave the second slope produced by the inner part of the cell unchanged.

Increasing Potassium Swells Endothelial Cells. AFM imaging of living endothelial cells reveals that cell height and cell volume increase when extracellular potassium is raised in a stepwise fashion from 4 mM to 6 mM and 8 mM (Fig. 2). Addition of barium to the bath solution containing 4 mM potassium initially swells the cells but prevents changes in cell volume when potassium is increased in the ambient solution. This experiment indicates that endothelial cells have active potassium channels in the plasma membrane that render the cell sensitive to changes in extracellular potassium concentration.

Potassium Modifies Endothelial Cell Stiffness. In several series of experiments, plasma potassium concentration was varied between 2 and 8 mM while ambient sodium was at either a low (130 mM) or high (150 mM) level. To study the effect of aldosterone, each series was divided into two groups, one with and one without the acute application of 0.45 nM aldosterone. The aldosterone series was performed in cells that had already been treated with aldosterone during their 48 h growth phase before the acute stiffness measurements. Fig. 3 shows the results. Clearly, the highest sensitivity to changes of potassium is observed in the absence of aldosterone when a low sodium permeability is expected (27% change from 4 mM to 6 mM potassium at 130 mM sodium; $P < 0.01$). There is nevertheless a decrease of cell stiffness even in the presence of aldosterone. This change in cell stiffness is dependent on the lower sodium concentration (17% change from 4 mM to 6 mM potassium at 130 mM sodium; $P < 0.01$). However, when sodium is increased to 150 mM and aldosterone is present, cortical cell stiffness reaches its highest value and now remains almost insensitive to changes of potassium (6% change from 4 mM to 6 mM potassium at 150 mM sodium; $P > 0.10$). Taken together, potassium

Author contributions: H.O., C.C., K.K.-V., V.S., G.A.M., and H.E.d.W. designed research; H.O. performed research; H.S. and C.R. contributed new reagents/analytic tools; H.O. analyzed data; and H.O. and H.E.d.W. wrote the paper.

The authors declare no conflict of interest.

Freely available online through the PNAS open access option.

[1]To whom correspondence should be addressed. E-mail: oberlei@uni-muenster.de.

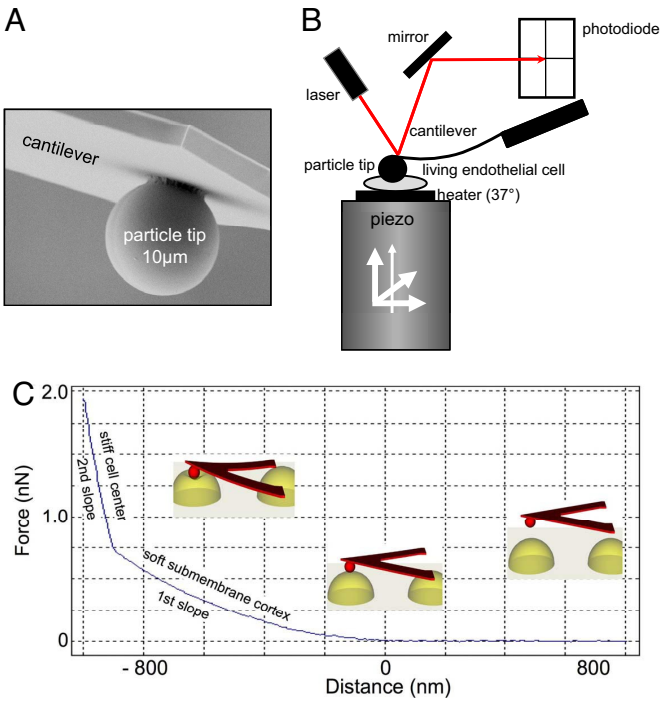

Fig. 1. Indentation technique using AFM. (*A*) Cantilever with particle tip. (*B*) Principle of the stiffness measurement using an AFM. (*C*) Indentation curve with two different slopes. After engagement of the cell surface with the particle tip, the cantilever will be bent as the cell is indented. A laser beam (as displayed in *B*; not shown in *C*) reflected from the cantilever quantifies this signal. From the two linear portions of the indentation curve, the cell stiffness can be calculated over the first few hundred nanometers of indentation (cortical cell stiffness) or beyond 800 nm of indentation (cell center).

softens cells more effectively when sodium level is low and aldosterone is absent.

Depolymerization of Cortical Actin by Cytochalasin D Softens Cells. Stiffness measurements on endothelial cells have shown that a low concentration of cytochalasin D (CD; range, ≤ 1 μM) can destabilize cortical actin (21). To test whether such a change in stiffness can be evoked in the GM7373 endothelial cells, CD was applied (1 μM) while cortical stiffness was measured. A large change in the first slope of the indentation curve became visible within minutes of CD application (Fig. 4). The changes are interpreted as being caused by softening and broadening of the cortical zone, while, in contrast, stiffness of the inner bulk of the cell did not change.

Potassium Has no Effect on Cortical Stiffness when Cortical Actin Is Destabilized. Fig. 5 shows the time course of the stiffness measurements after CD application and the lack of effect when potassium was then increased from 4 mM (initial potassium concentration in this experimental series) to 6 mM and 8 mM. In addition, this figure shows that the changes in stiffness are limited to the cell cortex because no changes consistent with a change at the center of the cell can be observed. Thus, only the outer zone of the cell is involved in the observed stiffness changes.

Activation of ENaC by Trypsin Stiffens Cells. A low concentration of the serine protease trypsin (range, ≤ 1 μg/mL) is known to activate functionally silent (i.e., inactive) ENaCs expressed in fibroblasts (22). To test whether facilitated sodium influx into the cell could change cortical stiffness, we applied 10 ng/mL trypsin while cortical stiffness was measured. Original indentation curves are shown in Fig. 4. After a delay of approximately 10 min, the first linear slope of the indentation curve shortened and grew steeper, indicating that the cortical zone became smaller and stiffer.

Amiloride Blocks the Trypsin-Mediated Increase in Cortical Stiffness. Trypsin (10 ng/mL) stiffens the submembranous cortical cell zone after a delay of approximately 10 to 15 min. Amiloride (1 μM), a selective blocker of ENaC, completely inhibits this response (Fig. 6). This indicates that activation of silent ENaC in the plasma membrane and Na^+ influx are responsible for the stiffness increase in the submembranous cortex.

Nitric Oxide Release Is Increased at High Potassium Levels. Nitric oxide synthase is localized beneath the plasma membrane in the submembranous cortex of endothelial cells (23). It is known that monomeric actin (G-actin) can serve as a stimulating protein of NO synthesis (24–26). As the submembranous cortex softens when potassium is increased and such a solation is likely caused by the transformation of polymeric actin (F-actin) into monomeric actin (G-actin), three series of experiments were conducted in which nitrite concentration in the supernatant culture medium was measured. GM7373 cells were cultured in flasks on a shaker that rhythmically tilted a thin layer of medium across the apical cell surface. The rhythmic movement of the medium across the cell surface was intended to mimic shear stress and thus raise NO synthesis to levels that can be reliably quantified (Fig. 7). In the first series of experiments, cells were cultured in low-sodium (135 mM) medium in the absence of aldosterone. In this series, nitrite concentration (used as an index of NO release into the medium) was found constant at extracellular potassium concentrations between 2 and 4 mM. A further increase to 6 and 8 mM, however, shifted nitrite concentration to significantly higher levels, indicating an increased release of NO into the supernatant. In a second series of experiments, 0.45 nM aldosterone was present in the (low-sodium) culture medium. As apparent from Fig. 7, nitrite formation was found significantly decreased at potassium concentrations of 2, 4, and 6 mM, most likely because of the presence of aldosterone in the medium. Furthermore, nitrite formation was unaffected by increases in potassium concentration between 2 and 6 mM. Only when potassium was increased further to 8 mM, significantly higher nitrite concentrations could be detected in the supernatant. In a third series of experiments, cells were cultured in high-sodium medium in the presence of aldosterone. Under these conditions, cells did not respond anymore to changes of extracellular potassium between 2 and 8 mM. Taken together, the findings suggest that a high potassium level can stimulate NO release as long as ambient sodium concentration is low, and aldosterone's effect on the entry of sodium into the cell again plays the role of a key modulator.

Discussion

Hypertension and cardiovascular disease are responsible for the greatest number of deaths worldwide (27). It is generally accepted that high sodium intake raises the arterial blood pressure whereas high potassium intake has the opposite effect (5, 7, 17–20). We have recently shown that a small physiological increase in extracellular sodium directly raises the stiffness of vascular endothelium and decreases its release of NO (16). This response is mediated by the ENaC. Here we report that an increase of extracellular potassium concentration significantly diminishes the stiffness of endothelial cells and improves the release of NO. Increases in potassium concentration to values such as those that occur during physical exercise in muscle, up to 12 mM (28, 29), or during neuronal activity in the brain (30), greatly soften endothelial cells. A similar softening of vascular endothelium has been shown recently when cells were are either exposed to estrogens (11) or to the β-blocker nebivolol, an antihypertensive agent that mimics the estrogen response in endothelial cells (31). Thus, the application of estrogens, nebivolol, and high potassium have two cellular responses in common:

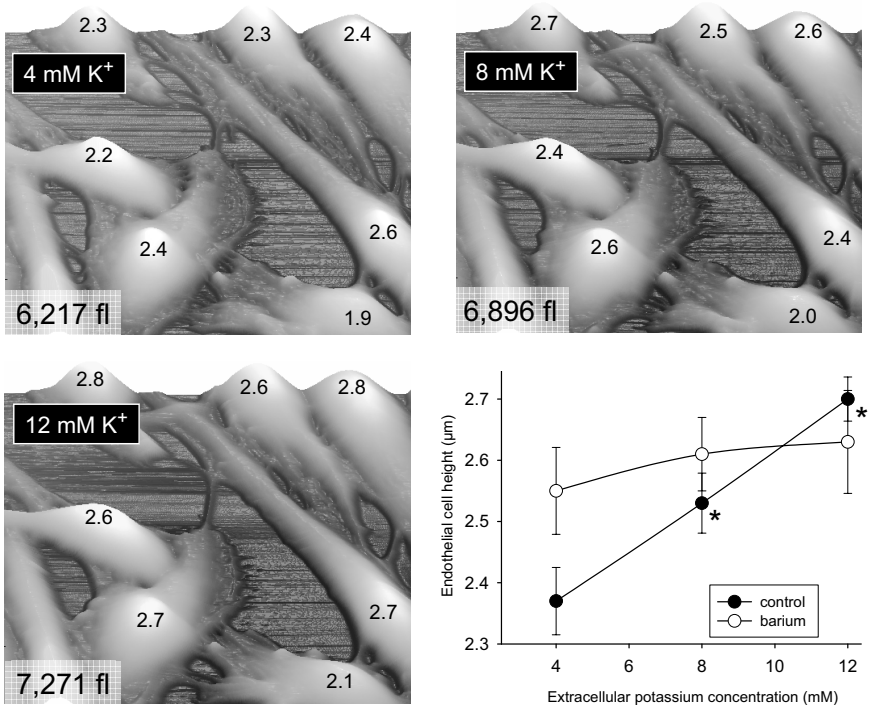

Fig. 2. AFM imaging of living vascular endothelial cells, exposed to increasing concentrations of extracellular potassium. Paired experiments showing the same cells at different conditions. Numbers on cells indicate the respective cell heights (in μm). Numbers (*Left*, *Lower*) refer to the total volume of the cells displayed. The graph shows the mean values of cell heights at the 3 different potassium concentrations in absence and presence of 3 mM barium (potassium channel block). Cells swell in response to increasing potassium. *Significant increase in cell height compared with the initial values measured at 4 mM potassium; $n = 7$, $P < 0.01$, paired Student *t* test. Barium prevents this response.

they swell and increase NO release. This swelling is different to the increase in cell size observed after aldosterone treatment (13, 14) when cells mainly increase their surface area, flatten, and stiffen (15). It is tempting to speculate that soft endothelial cells have a larger degree of physical compliance compared with stiff ones, and more easily undergo rapid morphological changes that occur during cardiac pulsations and thus generate more NO.

Ion-Driven Solation—Gelation Dynamics in the Cell Cortex. Cell types of diverse function are subjected to substantial stretch. For instance, upon physical stress, human airway smooth muscle cells promptly fluidize and then slowly re-solidify (32). Endothelial cells are subjected to large changes in cell shape (e.g., during dilation/constriction of blood vessels, particularly with each contraction of the heart) and can best adjust to such alterations if the deformability (i.e., physical compliance) of the cells is high.

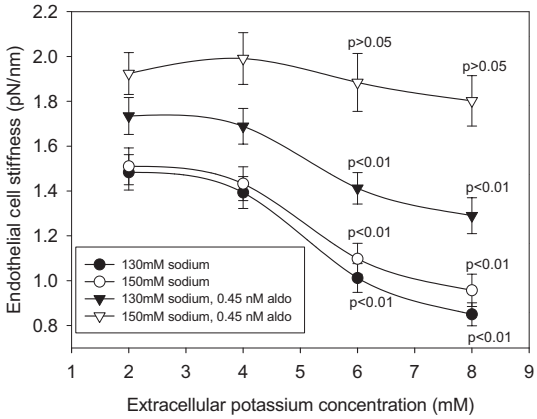

Fig. 3. Relationship between extracellular potassium concentration and cortical cell stiffness. Acute experiments were performed at four different conditions. In 2 series of experiments, aldosterone (*aldo*) was present in the perfusion solution (acute application) and in the culture medium 48 h before the experiment. Each series of experiments comprised 5 to 8 paired cell indentation measurements. The *P* values were calculated by comparing the individual mean values with the initial stiffness values at 2 mM potassium (paired Student *t* test).

We have defined two linear slopes in the indentation curves: the first tends to be flat whereas the second is steeper. The first flat slope indicates a low stiffness and is limited to the submembranous cortex of the cell. In agreement with recent force measurements applying an newly developed non-AFM method (33), there is a fluidic layer beneath the plasma membrane, which is highly dynamic in terms of thickness and viscosity. The cortical cytoskeleton of vascular endothelial cells is highly dynamic (34) and the state of polymerization of cortical actin determines the structure and mechanical properties of this layer (21, 35). Monomeric globular actin (G-actin) can rapidly polymerize into filamentous actin (F-actin), which should cause a rapid change in local viscosity. The cytochalasin experiments described here indicate that a destabilized actin that is switching from F-actin to G-actin is associated with solation of the cortex. Therefore, we propose that the increase in potassium softens the cortical actin cytoskeleton by changing F-actin to G-actin. G-actin is known to co-localize with the endothelial NO synthase (eNOS) and to increase endothelial NO synthase activity (24, 25). This matches well with the present observation that NO release from the endothelial cells into the medium is increased when extracellular potassium level is elevated.

Sodium is possibly a functional antagonist in this system. Sodium influx, as triggered in the present experiments by the activation of ENaC by aldosterone or trypsin, increases the viscosity of the submembranous layer and thus stiffens the cytoskeleton. When sodium is maintained in the upper physiological range, the high viscosity dominates, which prevents an

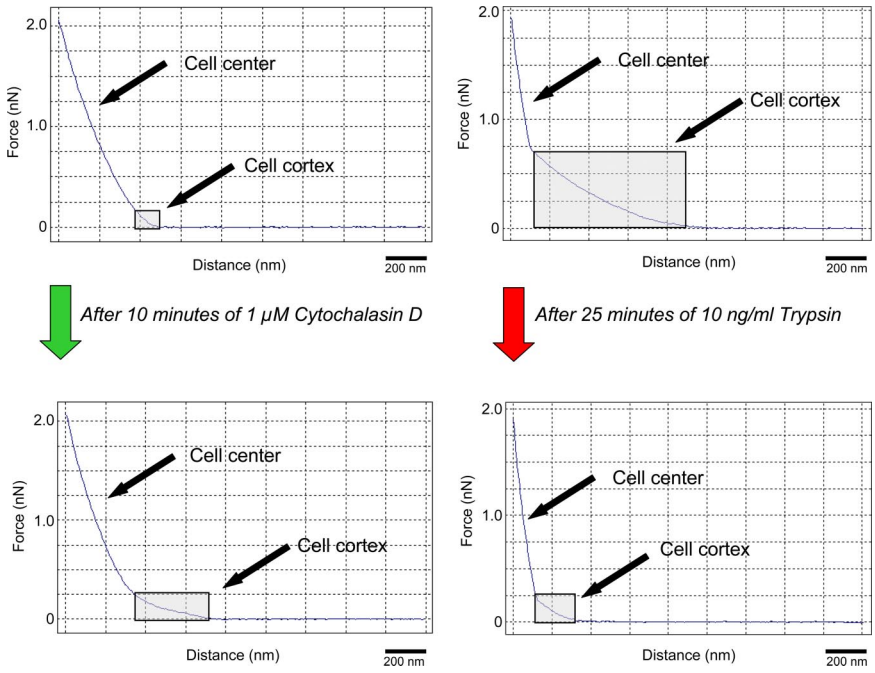

Fig. 4. Indentation curves obtained in 2 experiments on vascular endothelial cells. For better demonstration, 2 individual cells with a small (*Left*) and large (*Right*) initial cortical soft zone were chosen. *Left*, Before the application of CD, the first linear slope is short. It indicates that the cortical zone is shallow. Ten min after CD application, the cortical zone has broadened to several hundred nanometers. In addition, the first slope flattened, indicating that the cortical zone softened at the same time. *Right*, Before the application of trypsin, a broad and soft cortical zone is detected in this cell. Twenty-five min after addition of trypsin, this zone has shrunk. In addition, the rather steep slope indicates that the cortical zone not only shrunk but stiffened at the same time.

increase in potassium from having an effect. A possible explanation is that G-actin is a negatively charged protein and, as a result of the smaller hydration shell size of potassium compared with sodium (36), the affinity of G-actin for potassium is most likely greater than that for sodium.

Membrane Potential as a Possible Regulator of Cell Stiffness. In endothelial cells, the membrane potential is highly variable (37). One factor that may cause this variability is a change in potassium transport across the cell membrane. Starting at low extracellular potassium concentrations, an increase in potassium can paradoxically hyperpolarize the membrane potential, e.g., in endothelial and smooth muscle cells (38). Perhaps, therefore, a rapid change in membrane potential can directly or indirectly affect the state of polymerization of the submembrane cortical actin network.

There is a link between membrane potential and NO synthesis. Small- and intermediate-conductance calcium-activated potassium channels directly control NO synthesis in human vascular endothelial cells (39). Large-conductance calcium-activated potassium channels hyperpolarize vascular smooth muscle cells and thus decrease vascular tone. This channel type has been shown to be down-regulated in hypertension (40). As endothelial cells and smooth muscle cells are coupled via myo-endothelial gap junctions (41), electrical signals originating from either cell type will spread longitudinally and trans-vertically in the blood vessel and set the cell potentials in a specific vessel segment (42–44).

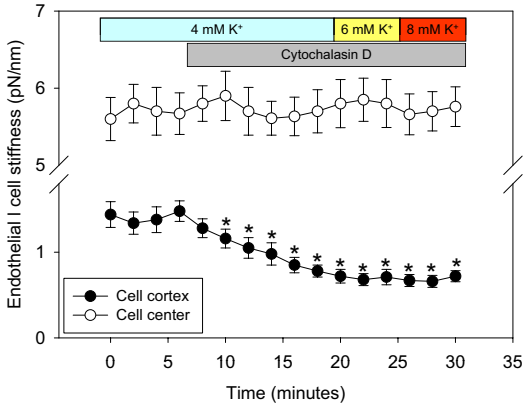

Fig. 5. Time course of the mean stiffness of 5 cells measured at 2 locations. After CD, the cortical cell stiffness decreases gradually while the stiffness in the cell center remains constant. Increasing extracellular potassium (from 4 mM to 6 mM and 8 mM) after the destabilization of the cortical actin does not further affect cortical stiffness. *Significant difference versus the respective initial value (paired Student *t* test).

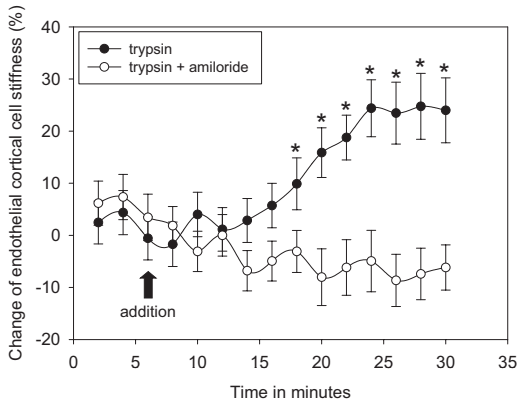

Fig. 6. Time course of cortical cell stiffness measurements (means of 4 cells) in response to trypsin. The increase of cortical cell stiffness can be prevented by 1 μM amiloride added at the same time together with trypsin. *Significant difference versus the respective initial value (paired Student *t* test).

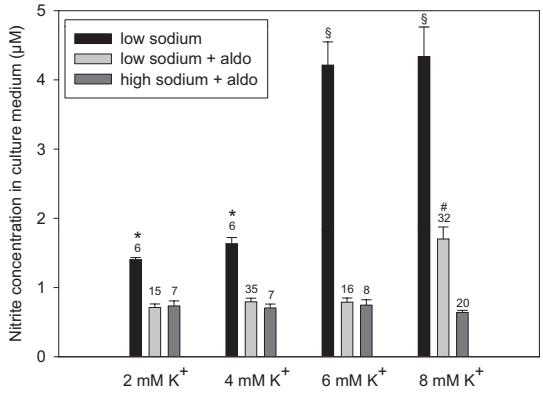

Fig. 7. Nitrite concentrations analyzed in the supernatant culture media after exposure of the vascular endothelial cells for 24 h to different potassium concentrations. Three series of experiments were performed (low sodium, low sodium plus aldosterone, high sodium plus aldosterone). The numbers on top of the individual columns indicate the number of nitrite measurements. Asterisk on top of a column indicates a significant difference compared with the mean values of the same group of experiments. §Significant difference versus all other mean values of the figure except those with the same symbol. #Significant difference versus respective mean values in the different groups of experiments. Significantly different is $P < 0.05$ (unpaired Student *t* test).

In contrast to potassium channels, any activation of ENaCs should depolarize the plasma membrane potential and thus switch G-actin to F-actin in the submembranous cell cortex. The present data support this view because activation of ENaC by trypsin (i.e., cell depolarization) stiffens the layer (most likely the cortical actin network) beneath the apical cell membrane and amiloride blocks this effect.

In conclusion, we have demonstrated that extracellular sodium and potassium, by different mechanisms, determine the physical compliance of endothelial cells and that aldosterone is an important modifier of this process. The ion-evoked changes in stiffness fluctuate between states of solation and gelation and are restricted to the submembranous cortex.

Methods

Endothelial Cell Culture. Bovine aortic endothelial GM7373 cells (DSMZ) were grown in culture as previously described (16). Briefly, confluent GM7373 cells (45) were cultured in T_{25} culture flasks using DMEM (Invitrogen) with addition of $NaHCO_3$, penicillin G, streptomycin (Biochrom), and 20% FBS (PAA Clone). After reaching confluence cells were split and then cultured on thin (diameter, 15 mm) glass coverslips. The coverslips were placed in Petri dishes filled with culture medium. GM7373 cells formed confluent monolayers within 48 h (at 37 °C, 5% CO_2).

Chemicals. Aldosterone (d-aldosterone; Sigma-Aldrich) was dissolved in ethanol (1 mM stock solution, stored at 4 °C for 2 weeks). Final concentration, measured in the culture medium (RIA, Adaltes) was 0.45 nM. Amiloride (Sigma-Aldrich), a selective blocking chemical of the ENaC, was dissolved in water, at a final concentration of 1 μM. CD (Sigma-Aldrich) was used to depolymerize the cortical actin cytoskeleton. Using AFM, destabilization of cortical actin can be detected at a concentration of 0.1 μM CD. CD was dissolved in buffered solution and a dose-response relationship obtained. A half-maximal change in cortical cell stiffness was noted 10 min after application of 0.5 μM CD. Therefore, a concentration of 1 μM CD was used, assuming this concentration to be low enough to attack only the cortical actin but to be sufficiently high to allow the detection of changes in stiffness using AFM. The serine protease trypsin (Sigma-Aldrich) was used to activate the ENaC (46). A concentration of 10 ng/mL trypsin was found to be sufficient to modify the AFM indentation curves and to detect the gelating effect of sodium.

Nitrite Concentration Measurements. Formation of NO in response to changes of extracellular potassium was determined from the accumulation of nitrite/nitrate (stable breakdown products of NO) in the culture medium (enriched by 250 μM L-arginine) of bovine aortic endothelial GM7373 cells (DSMZ). A slightly modified protocol than that published previously was used (16). In short, confluent GM7373 cells (45) were cultured in T_{75} flasks either in low-sodium medium (135 mM) or high-sodium medium (150 mM), in the presence or absence of 0.45 mM aldosterone. Initially, potassium-free medium was purchased (Invitrogen), to which potassium was added at a level between 2 and 8 mM. Osmolality was kept constant for all media, adding mannitol as appropriate. FBS was reduced from 20% to 5% to prevent protein interference during nitrite analysis. To compensate for the lack of macromolecules in the media, polyvinylpyrrolidone (40 kDa) was added to a final concentration of 35 g/L. Only 5.5 mL of medium was added to the individual culture flasks. Cultures were placed on a shaker inside the incubator and rhythmically shaken along the longitudinal flask axis (0.5 Hz). After 24 h, the harvested medium was centrifuged ($134 \times g$) and the supernatant was pressed through a 30-kDa exclusion filter (Amicon Ultracell 30 K; Millipore) by a 90 min centrifugation at $5{,}000 \times g$ at 18 °C. The filtrate (4 mL) was lyophilized and resuspended in 266 μL H_2O. Finally, the solution was mixed with Griess reagent and absorbance measured spectrophotometrically at 546 nm. Nitrite concentration was determined using a standard curve of known concentrations of $NaNO_2$ (1–200 μM). Stepwise filtering was necessary to remove proteins and polyvinylpyrrolidone from the solution. Lyophilization and subsequent re-suspension in a small volume (concentration factor was 15) shifted nitrite concentration to a better detection range. Rhythmic rocking of the culture flasks (i.e., rhythmic flooding of the cells with a small volume of medium) mimicked shear stress and raised NO formation to levels that could be reliably measured.

Endothelial Cell Volume and Stiffness Measurements. Volume of living GM373 cells was determined with AFM techniques as described previously (14, 15). Volume and stiffness of the endothelial cells were measured with soft cantilevers (MLCT-contact microlevers; spring constant, 0.01 N/m; Veeco). However, for stiffness measurements, a so-called colloidal probe tip was used (sphere diameter, 10 μm; Fig. 1). Colloidal probe tips are more suitable for cell stiffness measurements compared with measurements with sharp tips because the area of interaction between tip and cell is larger and thus mechanically less noisy (47). In principle, the AFM is used as a mechanical tool, i.e., the AFM tip is pressed against the cell so that the membrane is indented (Fig. 1). This distorts the AFM cantilever, which serves as a soft spring. The cantilever deflection, measured by a laser beam when reflected from the gold-coated cantilever, permits force-distance curves of single cells. The slope of such curves is directly related to the force (expressed in Newtons), defined here as stiffness, necessary to indent the cell.

Similar to previous investigations (21), two different slopes could be identified depending on the depth of indentation. The initial flat slope (indentation depth to several hundred nanometers) reflects the plasma membrane stiffness, including the cortical cytoskeleton, whereas the late steep slope reflects the stiffness of the cell center. Both slopes were analyzed and displayed.

Force-distance curves were obtained on single cells in paired fashion, i.e., potassium was increased stepwise while stiffness was measured at a rate of approximately 0.2 Hz in one individual cell. Measurements were performed on living cells at 37 °C using a feedback-controlled heating device (Veeco). The cells were bathed in Hepes buffered solution (standard composition in mM: 135 NaCl; 5 KCl; 1 $MgCl_2$; 1 $CaCl_2$; 10 Hepes, pH 7.4). Sodium (130–150 mM) and potassium (2–12 mM) concentrations were varied as appropriate. Iso-osmolality was obtained in all experiments by addition of mannitol if applicable.

Statistics. Data are shown as mean values ± SEM. Significance of differences was evaluated by the paired or unpaired Student *t* test if applicable. Overall significance level is $P = 0.05$.

ACKNOWLEDGMENTS. We thank Marianne Wilhelmi and Anja Blanque for their excellent work in endothelial cell culture. The study was supported by the German Research Council DFG OB63/17–1 and the EU project Tips4cells.

1. Roson MI, *et al.* (2006) Acute sodium overload produces renal tubulointerstitial inflammation in normal rats. *Kidney Int* 70:1439–1446.
2. Ni Z, Vaziri ND (2001) Effect of salt loading on nitric oxide synthase expression in normotensive rats. *Am J Hypertens* 14:155–163.
3. Mercier N, *et al.* (2007) Sodium, arterial stiffness, and cardiovascular mortality in hypertensive rats. *Am J Hypertens* 20:319–325.
4. Frohlich ED, Varagic J (2005) Sodium directly impairs target organ function in hypertension. *Curr Opin Cardiol* 20:424–429.
5. Adrogue HJ, Madias NE (2007) Sodium and potassium in the pathogenesis of hypertension. *N Engl J Med* 356:1966–1978.
6. Kido M, *et al.* (2008) Protective effect of dietary potassium against vascular injury in salt-sensitive hypertension. *Hypertension* 51:225–231.

7. He FJ, MacGregor GA (2001) Fortnightly review: beneficial effects of potassium. *BMJ* 323:497–501.
8. Khaw KT, Barrett-Connor E (1987) Dietary potassium and stroke-associated mortality. A 12-year prospective population study. *N Engl J Med* 316:235–240.
9. Tobian L, MacNeill D, Johnson MA, Ganguli MC, Iwai J (1984) Potassium protection against lesions of the renal tubules, arteries, and glomeruli and nephron loss in salt-loaded hypertensive Dahl S rats. *Hypertension* 6:I170–I176.
10. Rader DJ, Daugherty A (2008) Translating molecular discoveries into new therapies for atherosclerosis. *Nature* 451:904–913.
11. Hillebrand U, *et al.* (2006) 17beta-estradiol increases volume, apical surface and elasticity of human endothelium mediated by Na+/H+ exchange. *Cardiovasc Res* 69:916–924.
12. Gorelik J, *et al.* (2005) Aldosterone acts via an ATP autocrine/paracrine system: the Edelman ATP hypothesis revisited. *Proc Natl Acad Sci USA* 102:15000–15005.
13. Hillebrand U, *et al.* (2007) Dose-dependent endothelial cell growth and stiffening by aldosterone: endothelial protection by eplerenone. *J Hypertens* 25:639–647.
14. Kusche-Vihrog K, *et al.* (2008) Aldosterone and amiloride alter ENaC abundance in vascular endothelium. *Pflugers Arch* 455:849–857.
15. Oberleithner H, *et al.* (2006) Differential action of steroid hormones on human endothelium. *J Cell Sci* 119:1926–1932.
16. Oberleithner H, *et al.* (2007) Plasma sodium stiffens vascular endothelium and reduces nitric oxide release. *Proc Natl Acad Sci USA* 104:16281–16286.
17. He FJ, *et al.* (2005) Effect of short-term supplementation of potassium chloride and potassium citrate on blood pressure in hypertensives. *Hypertension* 45:571–574.
18. Fang Y, Mu JJ, He LC, Wang SC, Liu ZQ (2006) Salt loading on plasma asymmetrical dimethylarginine and the protective role of potassium supplement in normotensive salt-sensitive Asians. *Hypertension* 48:724–729.
19. Morris RC Jr, Sebastian A, Forman A, Tanaka M, Schmidlin O (1999) Normotensive salt sensitivity: effects of race and dietary potassium. *Hypertension* 33:18–23.
20. Haddy FJ, Vanhoutte PM, Feletou M (2006) Role of potassium in regulating blood flow and blood pressure. *Am J Physiol Regul Integr Comp Physiol* 290:R546–R552.
21. Kasas S, *et al.* (2005) Superficial and deep changes of cellular mechanical properties following cytoskeleton disassembly. *Cell Motil Cytoskeleton* 62:124–132.
22. Caldwell RA, Boucher RC, Stutts MJ (2004) Serine protease activation of near-silent epithelial Na+ channels. *Am J Physiol Cell Physiol* 286:C190–C194.
23. Ju H, Zou R, Venema VJ, Venema RC (1997) Direct interaction of endothelial nitric-oxide synthase and caveolin-1 inhibits synthase activity. *J Biol Chem* 272:18522–18525.
24. Kondrikov D, Han HR, Block ER, Su Y (2006) Growth and density-dependent regulation of NO synthase by the actin cytoskeleton in pulmonary artery endothelial cells. *Am J Physiol Lung Cell Mol Physiol* 290:L41–L50.
25. Su Y, Edwards-Bennett S, Bubb MR, Block ER (2003) Regulation of endothelial nitric oxide synthase by the actin cytoskeleton. *Am J Physiol Cell Physiol* 284:C1542–C1549.
26. Searles CD, Ide L, Davis ME, Cai H, Weber M (2004) Actin cytoskeleton organization and posttranscriptional regulation of endothelial nitric oxide synthase during cell growth. *Circ Res* 95:488–495.
27. Ezzati M, Lopez AD, Rodgers A, Vander HS, Murray CJ (2002) Selected major risk factors and global and regional burden of disease. *Lancet* 360:1347–1360.
28. Mohr M, *et al.* (2004) Potassium kinetics in human muscle interstitium during repeated intense exercise in relation to fatigue. *Pflugers Arch* 448:452–456.
29. Nordsborg N, *et al.* (2003) Muscle interstitial potassium kinetics during intense exhaustive exercise: effect of previous arm exercise. *Am J Physiol Regul Integr Comp Physiol* 285:R143–R148.
30. Kofuji P, Newman EA (2004) Potassium buffering in the central nervous system. *Neuroscience* 129:1045–1056.
31. Hillebrand U, *et al.* (2009) Nebivolol decreases endothelial cell stiffness via the estrogen receptor beta: a nano-imaging study. *J Hypertens*, in press.
32. Trepat X, *et al.* (2007) Universal physical responses to stretch in the living cell. *Nature* 447:592–595.
33. Sanchez D, *et al.* (2008) Non-contact measurement of the local mechanical properties of living cells using pressure applied via a pipette. *Biophys J* 95:3017–3027.
34. Pesen D, Hoh JH (2005) Modes of remodeling in the cortical cytoskeleton of vascular endothelial cells. *FEBS Lett* 579:473–476.
35. Pesen D, Hoh JH (2005) Micromechanical architecture of the endothelial cell cortex. *Biophys J* 88:670–679.
36. Joseph NR, Engel MB, Catchpole HR (1961) Distribution of sodium and potassium in certain cells and tissues. *Nature* 191:1175–1178.
37. Nilius B, Droogmans G (2001) Ion channels and their functional role in vascular endothelium. *Physiol Rev* 81:1415–1459.
38. Edwards G, Dora KA, Gardener MJ, Garland CJ, Weston AH (1998) K+ is an endothelium-derived hyperpolarizing factor in rat arteries. *Nature* 396:269–272.
39. Sheng JZ, Braun AP (2007) Small- and intermediate-conductance Ca2+-activated K+ channels directly control agonist-evoked nitric oxide synthesis in human vascular endothelial cells. *Am J Physiol Cell Physiol* 293:C458–C467.
40. Amberg GC, Bonev AD, Rossow CF, Nelson MT, Santana LF (2003) Modulation of the molecular composition of large conductance, Ca(2+) activated K(+) channels in vascular smooth muscle during hypertension. *J Clin Invest* 112:717–724.
41. Sandow SL, Tare M (2007) C-type natriuretic peptide: a new endothelium-derived hyperpolarizing factor? *Trends Pharmacol Sci* 28:61–67.
42. Griffith TM, Chaytor AT, Edwards DH (2004) The obligatory link: role of gap junctional communication in endothelium-dependent smooth muscle hyperpolarization. *Pharmacol Res* 49:551–564.
43. Chaytor AT, Edwards DH, Bakker LM, Griffith TM (2003) Distinct hyperpolarizing and relaxant roles for gap junctions and endothelium-derived H2O2 in NO-independent relaxations of rabbit arteries. *Proc Natl Acad Sci USA* 100:15212–15217.
44. Griffith TM, Chaytor AT, Taylor HJ, Giddings BD, Edwards DH (2002) cAMP facilitates EDHF-type relaxations in conduit arteries by enhancing electrotonic conduction via gap junctions. *Proc Natl Acad Sci USA* 99:6392–6397.
45. Grinspan JB, Mueller SN, Levine EM (1983) Bovine endothelial cells transformed in vitro by benzo (a) pyrene. *J Cell Physiol* 114:328–338.
46. Jovov B, Berdiev BK, Fuller CM, Ji HL, Benos DJ (2002) The serine protease trypsin cleaves C termini of beta- and gamma-subunits of epithelial Na+ channels. *J Biol Chem* 277:4134–4140.
47. Carl P, Schillers H (2008) Elasticity measurement of living cells with an atomic force microscope: data acquisition and processing. *Pflugers Arch* 457:551–559.