Aldosterone remodels human endothelium

H. Oberleithner,[1] C. Riethmüller,[1] T. Ludwig,[2] M. Hausberg[3] and H. Schillers[1]

1 Institute of Physiology II, University Münster, Germany

2 Department of Cellular and Molecular Physiology, Yale University Medical School, New Haven, CT, USA

3 Department of Internal Medicine D, University Münster, Germany

Received 23 September 2005, accepted 23 January 2006
Correspondence: H. Oberleithner, Institut für Physiologie II, Robert-Koch-Str. 27b, 48149 Münster, Germany.
E-mail: oberlei@uni-muenster.de

Abstract

Aim: In response to aldosterone endothelial cells swell and stiffen. Although amiloride-sensitive sodium and water uptake is known to be involved, the underlying mechanisms are yet unclear. We tested the hypothesis whether the intracellular accumulation of water or organic matter is responsible for the structural and functional alterations.

Methods: Atomic force microscopy was used as an imaging tool and a mechanical nanosensor. Cell water, organic cell matter and cell pressure was measured at single cell level in human umbilical vein endothelial cells (HUVEC). Furthermore, we tested by means of a miniature perfusion chamber *in vitro* the physical robustness to blood flow of the aldosterone-treated endothelium.

Results: In response to a three-day treatment with 1 nM aldosterone HUVEC swell. To our surprise, cell water decreased from 82 ± 6% to 71 ± 5% while intracellular organic matter increased from 18 ± 1.8% to 29 ± 3.0%. These changes were paralleled by a rise in cell pressure of 114%, measured in living HUVEC *in vitro*. Blood flow across the endothelium was found significantly altered after aldosterone treatment. Imaging the endothelial monolayer after blood perfusion disclosed large gaps between cells treated with aldosterone. The mineralocorticoid receptor blockers, spironolactone and eplerenone could prevent the aldosterone actions.

Conclusion: Mild aldosteronism causes intracellular accumulation of organic matter at the cost of cell water. This makes endothelium stiff and vulnerable to shear stress. The measurements could explain clinical observations that high blood pressure combined with high plasma aldosterone concentration may damage the endothelium of blood vessels.

Keywords aldosteronism, atomic force microscopy, cell water, eplerenone, mineralocorticoid, spironolactone.

Systemic blood pressure is clearly under the control of aldosterone. The so far unchallenged concept is the hormone's salt and water retaining action in kidney. However, clinical studies strongly indicate that the cardiovascular system is another target tissue for mineralocorticoids (Mendelsohn 2005). Alarmed by the impressive clinical findings physiologists started to investigate the mechanisms underlying aldosterone action in blood vessels. Some striking similarities in aldosterone action were found between renal collecting duct and endothelium. Both structures express mineralocorticoid receptors and plasma membrane epithelial sodium channels (Golestaneh *et al.* 2001). Endothelial cells swell in response to aldosterone due to amiloride-sensitive sodium uptake across the apical membrane (Oberleithner *et al.* 2004). Applying high aldosterone

 doi: 10.1111/j.1748-1716.2006.01574.x

concentrations (10 nM) cells develop high intracellular pressure and exhibit, at least *in vitro*, altered monolayer formation (Oberleithner 2005).

The goal of the present study was to disclose the mechanism of the cell pressure increase, mediated by a physiological concentration of aldosterone and its potential relevance for endothelial barrier function. The focus was put upon changes in cell function caused by chronic (three-day) treatment with aldosterone. The atomic force microscope was used in two different applications, as an imaging tool and as a mechanical sensor for single cell pressure measurements (Henderson & Oberleithner 2000). We imaged human umbilical vein endothelial cells cultured in appropriate media in 'wet' and 'dry' conditions to obtain information on cell water content. Furthermore, we measured cell pressure for the first time at 37 °C *in vitro*. Finally, we shear-stressed endothelial monolayers with human whole blood using a miniature perfusion and analysed the endothelial barrier after perfusion.

Methods

Endothelial cell culture

Human umbilical venous endothelial cells (HUVECs) were grown in culture as previously described (Jaffe *et al.* 1973, Goerge *et al.* 2002). In brief, cells (passage p0) were cultivated in T_{25} culture flasks coated with 0.5% gelatine (Sigma–Aldrich Chemie GmbH, Steinheim, Germany). After reaching confluence, cells were split using trypsin and then cultured (passage p1) on thin (diameter = 15 mm) glass coverslips coated with 0.5% gelatine and cross-linked with 2% glutaraldehyde. Glass coverslips were placed in Petri dishes filled with culture medium. HUVEC formed confluent monolayers within 72 h (at 37 °C, 5% CO_2). Chemicals were added to the medium as appropriate. Aldosterone (d-aldosterone, Sigma–Aldrich Chemie GmbH, Steinheim, Germany) was dissolved in ethanol (1 mM stock solution, stored at 4 °C for 2 weeks). Final concentration in the experiments was 1 nM. Spironolactone (ICN Biochemicals GmbH, Eschwege, Germany) was dissolved in ethanol (1 mM stock solution) and applied with a final concentration of 10 nM. Eplerenone (SC-66110; Pfitzer Corp, Kalamazoo, MI, USA) was dissolved in ethanol (2 mM stock solution, stored at −20 °C). Final concentration in the experiments was 2 μM. Finally, we used the epithelial Na^+ channel blocker amiloride (Sigma–Aldrich), dissolved in water, at a final concentration of 0.1 μmol L^{-1}. In corresponding control experiments we added only the solvents at appropriate concentrations to the media. If applicable, HUVECs were exposed to glutaraldehyde (0.5% final concentration), gently added to the medium. In one series of experiments, we stored the fixed cells in HEPES buffered solution and investigated them under 'wet' conditions (cells scanned in fluid). In another series, we washed the cells with distilled water, dried them at room temperature overnight and then investigated them under 'dry' conditions (cells scanned in air).

AFM imaging

The method of three-dimensional cell imaging by AFM has been described previously (Henderson *et al.* 1996, Schneider *et al.* 2004). With fixed cells two subsequent series of AFM experiments were carried out: The first series was performed in fluid (HEPES buffered solution in mM: 140 NaCl; 5 KCl; 1 $MgCl_2$; 1 $CaCl_2$; 10 HEPES (N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid); pH = 7.4) to obtain total cell volume. The second series was performed in air using the same samples after a 'wash and dry' procedure to obtain the 'water-free' volume of the cells. A Nanoscope IIIa Multimode-AFM (VEECO, Mannheim, Germany) equipped with a V-shaped DNP-S gold-coated cantilever (spring constant about 0.06 N m^{-1}) was used for scanning.

Surface profiles (512 x 512 pixels) were obtained with scan sizes of 10 000 μm^2 at a scan rate of 6 Hz. Ten three-dimensional images were obtained from individual samples and endothelial cell volume was determined in fluid and air (i.e. wet and dry state, respectively), according to methods described in detail previously (Schneider *et al.* 2004).

AFM cell pressure measurements

Measurements of the elastic modulus (given in kPa and termed 'cell pressure') in living HUVECs, cultured under various conditions, were performed with AFM using the same equipment, as described above, except that softer cantilevers were used (MLCT-contact microlevers, spring constant: 0.01 N m^{-1}; Digital Instruments). Technical details have been published previously (Schneider *et al.* 2004). In principle, the AFM is used as a pressure sensor. The AFM tip is pressed against the cell so that the membrane is indented. At the same time, the AFM cantilever that serves as a soft spring is distorted. Force–distance curves quantify the force necessary to indent the membrane for a given distance. Force–distance curves were made on individual cells identified by AFM in the HUVEC monolayer. The elastic (Young's) modulus was estimated using the Hertz model that describes the indentation of elastic material (Radmacher *et al.* 1996), defined as follows: $F = \delta^2 \times (2/\pi) \times [E/(1 - v^2)] \times \tan \alpha$, where F is the applied force (calculated from the spring constant (0.01 N m^{-1}) multiplied by the measured cantilever deflection), E is the elastic modulus (kPa), v is the

  doi: 10.1111/j.1748-1716.2006.01574.x

Poisson's ratio assumed to be 0.5 because the cell is considered incompressible, α is the opening angle of the AFM tip (35°) and δ is the indentation depth (300 nm). Intracellular pressure measurements were performed in living cells at 37 °C using a feedback controlled heating device (Digital Instruments). During the measurements, HUVEC were bathed in HEPES buffered solution (see above). It should be mentioned that hormone and/or drug treatment occurred prior the experiments when cells were cultured in the respective media and that all 'out of the incubator' measurements were performed in HEPES buffered saline without any other additives.

Blood flow experiments

The so-called 'fluid chamber' of the AFM multimode, a commercially available perfusion chamber (Digital Instruments), was used to measure blood flow across a HUVEC monolayer in vitro at well-defined conditions (constant hydrostatic pressure, 37 °C). The principle of the measurement and some technical aspects are shown in Figure 1. The experimental procedure was as follows: In a first step, a HUVEC monolayer grown on gelatine coated glass was inserted in the miniature chamber (chamber volume about 15 μL). Then, in a second step, HEPES buffered electrolyte solution was perfused at constant hydrostatic pressure (9 kPa) and the flow rate was set to 150 μL per second. In a third step, heparinized whole blood from healthy donors was added and perfusion rate measured in 10 s intervals over the next two minutes. Whole blood as a highly viscous inhomogeneous solution was used to challenge the endothelium with significant shear stress. In a fourth step, the superfused HUVEC monolayer was gently rinsed with buffered saline again outside the perfusion chamber and then fixed in 0.5% glutaraldehyde dissolved in buffer. In a sixth and final step, the HUVECs were imaged with the AFM in buffered saline.

Statistics

For each experimental series endothelial cells obtained from one individual umbilical cord were used. Data of experiments are given as mean values (±SEM). Significance was tested employing analysis of variance with *post-hoc* tests (Fisher's PLSD) if allowed or the Kruskal–Wallis-test if normal distribution failed. Results were considered to be statistically significant when $P < 0.05$.

Results

Figure 2 compares images of HUVEC either scanned in buffered solution or in air. For comparison, dimensions in x, y and z directions were the same. Two differences are obvious: First, as known from previous studies (Oberleithner *et al.* 2003) cells treated for 3 days with aldosterone grow in size. Second, the dried preparation clearly shows intracellular features as dimples and depressions that are missing in the wet preparation. This was expected since in cells maintained in buffer all intracellular compartments are filled with fluid while after dehydration only organic cell matter remains in place. Only cell water is lacking under the latter conditions. Such images can be quantitatively analysed and volume per cell can be calculated. Figure 3 shows the mean data. They confirm previous experiments that cells, in comparison to controls (1294 ± 79 fl), significantly swell in presence of aldosterone (1661 ± 65 fl), an effect inhibited by the mineralocorticoid inhibitor spironolactone (1330 ± 82 fl). A difference to previous studies (Oberleithner *et al.* 2003) is that in the present series of experiments we used 10 times lower hormone

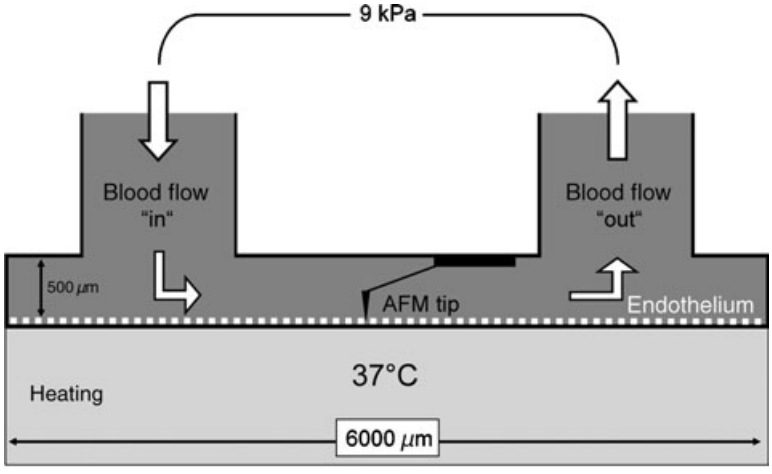

Figure 1 Set up of endothelial perfusion. A miniature perfusion chamber (called 'fluid cell') is mounted on the stage of an atomic force microscope (AFM). The chamber is used to perfuse a human endothelial monolayer with whole blood at defined pressure and temperature. Blood flow rate is measured as a function of time.

 doi: 10.1111/j.1748-1716.2006.01574.x

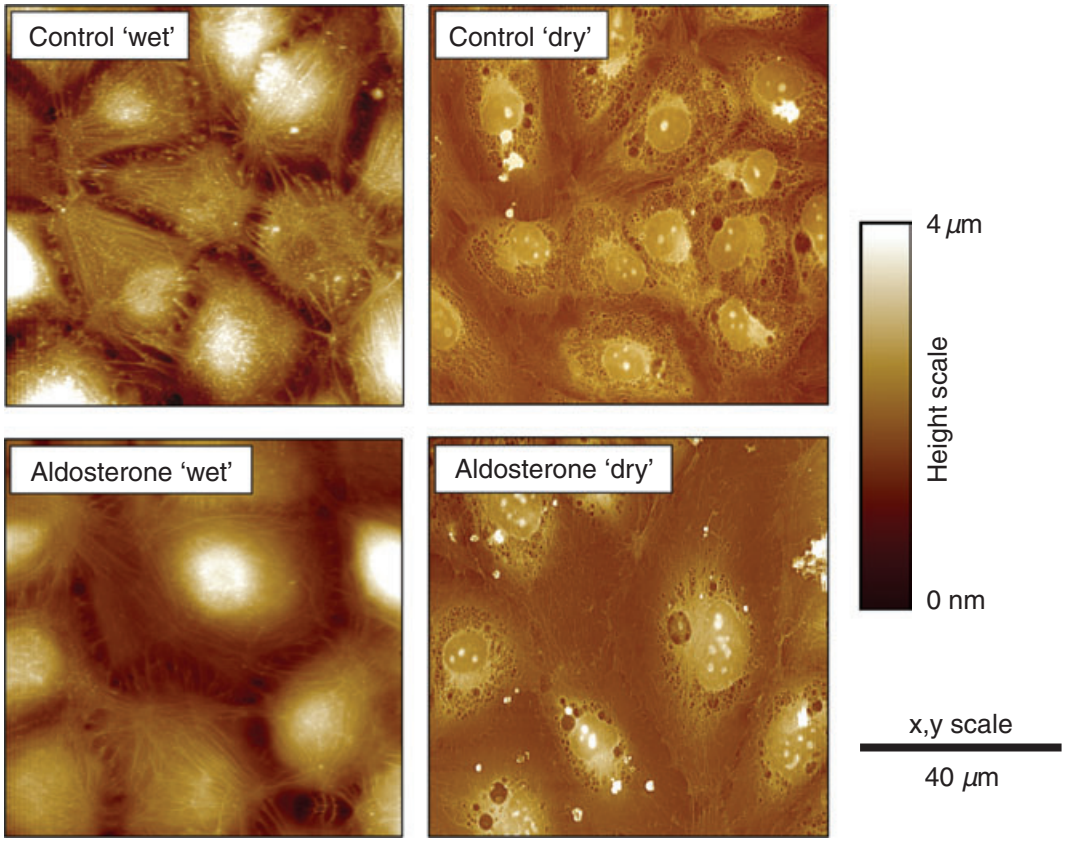

Figure 2 Top view AFM images of human endothelial cells (HUVEC) grown for 3 days in absence (control) or presence of 1 nM aldosterone. Cells fixed with glutaraldehyde were either scanned in fluid ('wet') or in air ('dry').

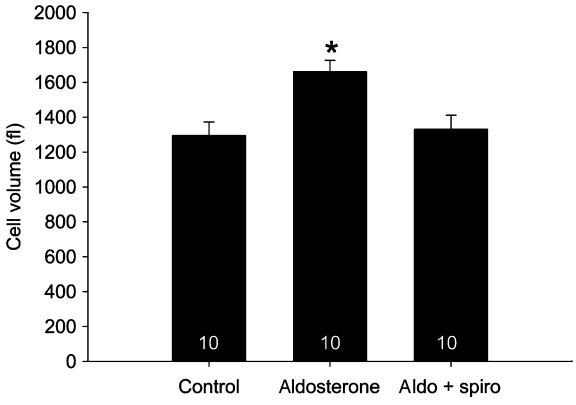

Figure 3 Volume of individual endothelial cells (HUVEC). Cells were exposed for 3 days either to the solvent (control), 1 nM aldosterone (aldo) and 1 nM aldosterone + 10 nM spironolactone (aldo + spiro). Cells were scanned in fluid ('wet'). Mean values ± SEM (number of observations in columns). *Indicates significant difference in comparison with control.

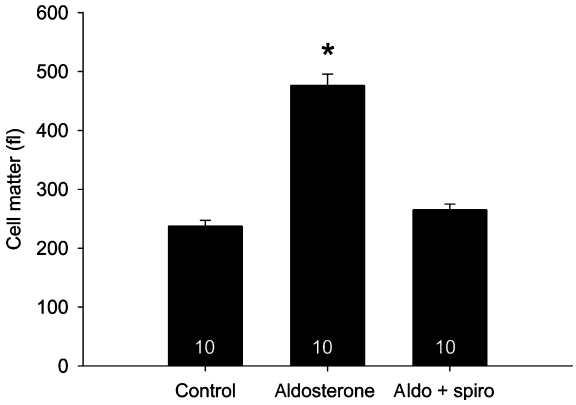

Figure 4 Cell matter of individual endothelial cells (HUVEC). Cells were exposed for 3 days either to the solvent (control), 1 nM aldosterone (aldo) and 1 nM aldosterone + 10 nM spironolactone (aldo + spiro). Cells were scanned in air ('dry'). Mean values ± SEM (number of observations in columns). *Indicates significant difference in comparison with control.

concentrations. The data indicate that the cells still respond. Figure 4 represents the mean data of cell volume after dehydration. There is a sharp increase in cell matter upon aldosterone treatment (from 237 ± 10.1 to 476 ± 19.4 fl). Again, this change in cell matter can be completely inhibited by spironolactone (265 ± 9.8 fl). In a next step we calculated cell water and cell matter in relative terms (Fig. 5). In absence of aldosterone (control) HUVEC consist of

  doi: 10.1111/j.1748-1716.2006.01574.x

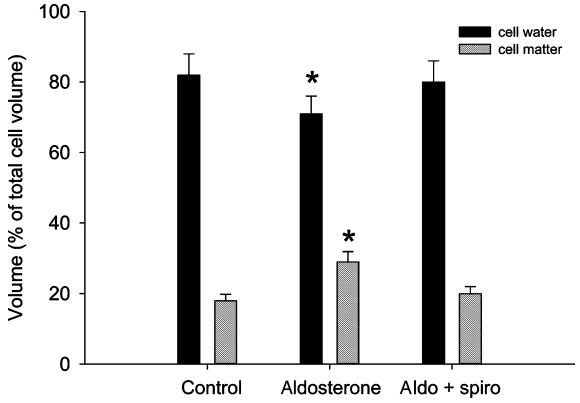

Figure 5 Cell water and cell matter expressed in percent of total cell volume of individual endothelial cells (HUVEC). *Indicates significant difference in comparison with corresponding control values ($n = 10$). Please note that the sum of cell water and cell matter amounts to cell volume, which is given in Figure 3.

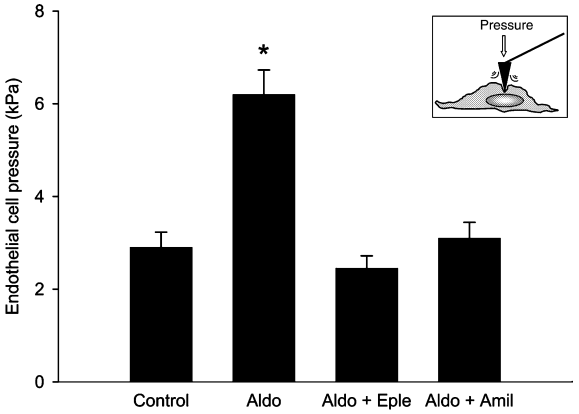

Figure 6 Cell pressure in living HUVEC. Cells in culture were maintained for 3 days in control conditions (control), in presence of 1 nM aldosterone (aldo), 1 nM aldosterone + 2 μM eplerenone (aldo + eple) and 1 nM aldosterone + 0.1 μM amiloride (aldo + amil). Inset in figure shows cell indentation by the mechanical sensor. Mean values ± SEM (number of pressure measurements (n) per series of experiments = 10). Cell pressure after aldosterone treatment is significantly different from the other mean values (indicated by *).

82 ± 6% water. Chronic aldosterone treatment significantly reduces cell water to 71 ± 5%, an effect prevented by spironolactone (80 ± 6%). A mirror-like image was obtained when relative cell matter is considered. There is a significant rise in cell matter from 18 ± 1.8% (control) to 29 ± 3.0% (aldosterone), again inhibited by spironolactone (20 ± 2.1%). Although cell pressure measurements have been reported previously (Oberleithner 2005), they have neither been performed with low aldosterone concentrations, nor the more selective aldosterone receptor blocker, eplerenone, was tested in those experiments. In addition, we did another series of experiments adding the plasma membrane sodium channel blocker amiloride in a low concentration (0.1 μM) to the incubation medium to test the hypothesis that a reduced influx of sodium could antagonize the hormone's response. Figure 6 shows the results. Aldosterone significantly increases cell pressure from 2.9 ± 0.33 (control) to 6.2 ± 0.54 kPa. Eplerenone as well as amiloride, present in the incubation media could prevent the pressure increase completely (2.4 ± 0.27 and 3.1 ± 0.31, respectively).

Finally, we performed a series of experiments to disclose any functional impact of the change in cellular water content and increase in cellular pressure. Previous studies indicated that chronic aldosterone treatment causes multiple gap formation between cells (Oberleithner 2005). Therefore, we hypothesized that structural changes of the endothelial surface mediated by aldosterone could have some impact to blood flow. We tested this hypothesis with a perfusion system in vitro. The results are shown in Figure 7. The rate of perfusion of whole blood at constant hydrostatic pressure through a narrow compartment (height = 500 μL) was measured every 10 s. The bottom of the perfusion chamber

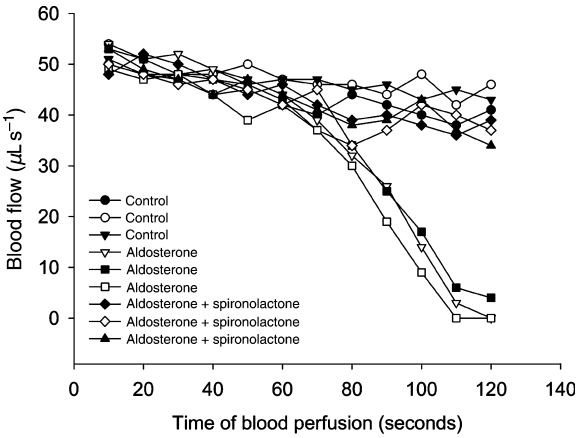

Figure 7 Blood flow as a function of time through the miniature perfusion chamber in which a HUVEC monolayer is mounted. Prior to the experiments cells were exposed for 3 days either to solvent (control), 1 nM aldosterone or 1 nM aldosterone + 10 nM spironolactone (three experiments for each condition).

was formed by a HUVEC monolayer. Using a control HUVEC preparation (no aldosterone added to the medium during the 3 days incubation prior to this experiment) a gradual decrease of flow rate is measured over a range of 2 min. However, the perfusion rates measured on monolayers treated with aldosterone sharply declined 70 s after blood perfusion was started and approached a complete stop of perfusion 40 s later. After blood perfusion, we dissembled the miniature chamber, rinsed the monolayers and fixed them. AFM

 doi: 10.1111/j.1748-1716.2006.01574.x

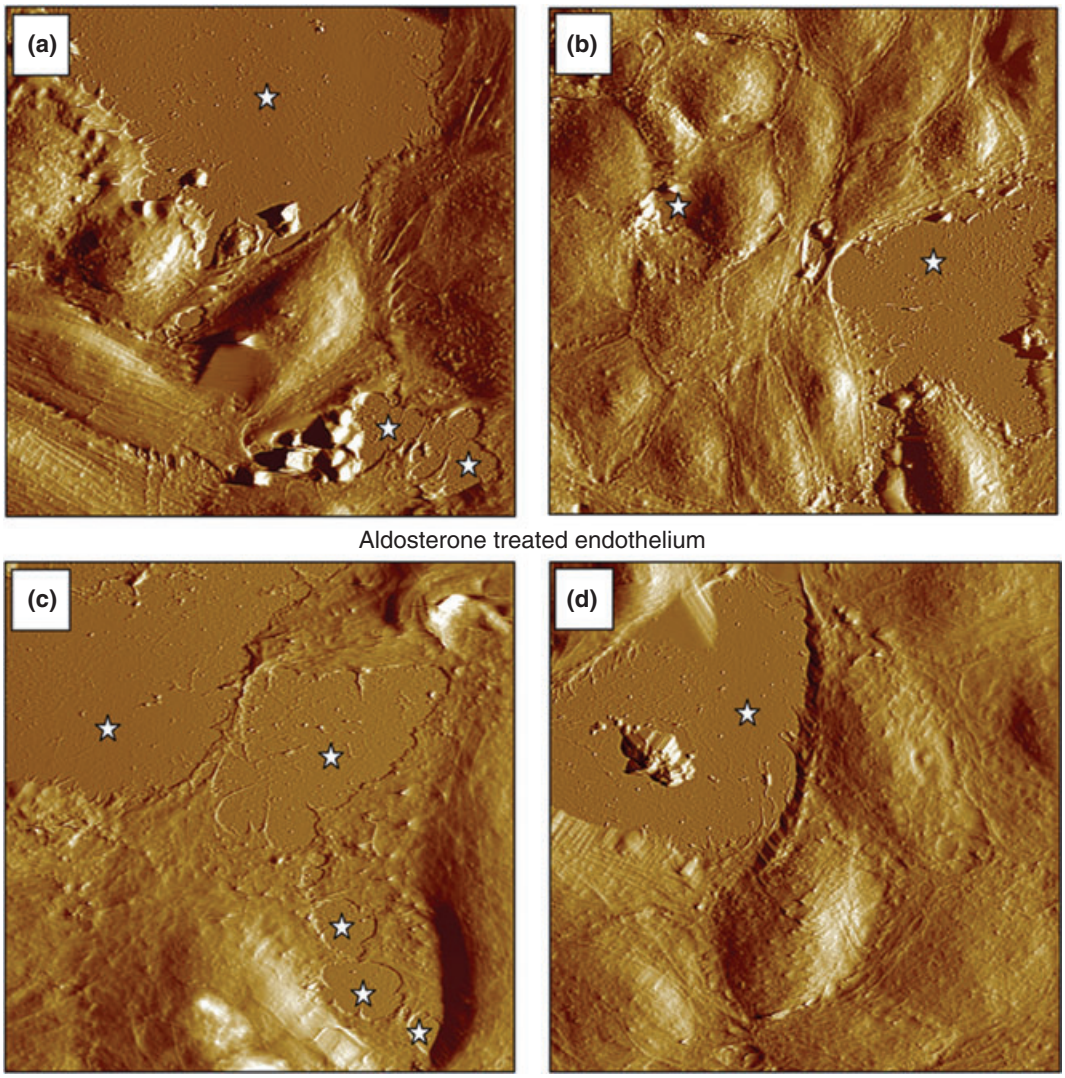

Figure 8 Top view AFM images of HUVEC monolayers grown for 3 days in presence of 1 nM aldosterone and then perfused with blood. Please note the large gaps between cells (indicated by stars). Gaps indicate lack of cells. Images sizes: $A = 70 \times 70\ \mu m^2$; $B = 100 \times 100\ \mu m^2$; C and $D = 50 \times 50\ \mu m^2$.

imaging of these monolayers revealed multiple gaps in the monolayer. Figure 8 shows four examples. In fact, individual cells in the monolayer are missing and the gelatine surface is exposed. This could not be observed in HUVEC monolayers grown in the absence of aldosterone (data not shown).

Discussion

Aldosterone controls fluids and electrolytes in the human organism. The major target is the kidney, where the collecting ducts are under the control of this hormone. There salt is selectively reabsorbed and water retained in order to maintain physiological body function. There is accumulating evidence that the cardiovascular system is another potentially important target structure for aldosterone. Specific mineralocorticoid actions could be shown in heart and blood vessels (Stier *et al.* 2002). We recently noticed that vascular endothelium and renal epithelium have some striking similarities (Oberleithner 2004). Cells express mineralocorticoid receptors and epithelial sodium channels (Golestaneh *et al.* 2001). Similar to renal target cells endothelial cells respond to diuretics.

One of the visible responses to aldosterone is cell swelling. At first sight this response is not surprising. Uptake of salt and water mediated by apically expressed epithelial sodium channels (Golestaneh *et al.* 2001) could easily explain the increase in cell size. This view is strongly supported also by the inhibitory action of amiloride, a sodium channel blocker. Moreover, mechanical measurements at single cell level point in a

  doi: 10.1111/j.1748-1716.2006.01574.x

similar direction, namely that cell pressure rises in response to the hormone. However, some recent observations in our laboratory indicate that an increase in cell volume may not be necessarily accompanied by an increase in cell pressure. Another class of steroid hormones, estrogens, swell endothelial swells even more than reported for aldosterone but, at the same time, cell pressure substantially decreases (unpublished observation in our laboratory). The question turned up whether aldosterone indeed changes the water content of a target cell or rather the solid cell components.

To address this question, we developed a method to measure cell water and cell matter (includes all organic material) at single cell level. We fixed cells under appropriate conditions and measured the respective volumes in presence and absence of cell water. Atomic force microscopy is the most suitable method for such an approach, for several reasons: (i) Any structures with heights in the range of up to about 12 μm, adherent to a solid surface, can be imaged at three dimensions and volumes quantified. (ii) Images can be obtained in fluid and in air. (iii) Fixed cells can be used so that the organic contents of a cell remain in place while water can be removed if desired. (iv) No surface coating is necessary that could influence the quantitative data.

To our surprise, we did not find an increase in cell water mediated by aldosterone. In contrary, we found a substantial increase in organic cell matter at the cost of cell water. The sequence of events triggered by aldosterone could be as follows: upon application of the hormone there is a fast non-genomic response. This could comprise the influx of inorganic ions across plasma membrane (Gekle *et al.* 1996, Schneider *et al.* 1997, Maguire *et al.* 1999). Already at this early stage of mineralocorticoid action classical intracellular receptors could be involved (Grossmann *et al.* 2005). Endothelial cell swelling observed already 5 min after application of the steroid, can be completely prevented by the aldosterone receptor blocker spironolactone (Oberleithner *et al.* 2003). The fact that the epithelial sodium channel blocker amiloride is also able to prevent swelling strongly indicates that sodium influx is closely related to the volume increase. As previously reported endothelial cells perform a volume regulatory decrease some 20 min later (Oberleithner *et al.* 2003). From studies in oocytes, it can be derived that within this first 20 min of aldosterone exposure the cell's genome is already activated leading to the export of mRNA from the cell nucleus (Schafer *et al.* 2002). This most likely indicates that the whole machinery of *de novo* protein synthesis has been started. With this initial scenario in mind, it is not surprising that cells grow in size (volume) when aldosterone exposure is maintained over days. It emphasizes the proliferative character of this hormone.

With atomic force microscopy techniques, we measured endothelial cell pressure. Although such measurements have been performed with similar approaches previously (Mathur *et al.* 2001, Kataoka *et al.* 2002), the present study reports for the first time measurements on cell pressure at 37 °C. Cell pressure was found more than doubled after 3 days of aldosterone treatment. In view of the present data that cell water is substituted by organic matter, we conclude as follows: Aldosterone-induced de novo synthesis increases macromolecular crowding. This has important impact upon form and function of intracellular macromolecules (Garner & Burg 1994). With the increase in the absolute number of intracellular macromolecules per cell colloid-osmotic pressure will also rise. This could explain the observed pressure increase. However, from the technical point of view we cannot distinguish between changes in cell pressure and changes in cell structure. Intracellular macromolecules, augmented in number by aldosterone, could be used to fortify the cytoskeleton. Then, single macromolecules (e.g. actin monomers) polymerize and stiffen the cell. In other words, so far we cannot distinguish between changes in pressure and changes in structure. Both changes could occur in response to aldosterone. Nevertheless, as a result of aldosterone treatment endothelial cells stiffen. This has potentially important impact on its function as obvious from the shear stress experiments of this study. Using identical experimental conditions *in vitro* an endothelial monolayer is less robust to a blood stream when preincubated with 1 nM aldosterone. Such a concentration would reflect mild aldosteronism in the human. We do not yet know the underlying mechanism of this vulnerability. As discussed above, it could be the consequence of an elevated intracellular pressure or a too rigid cytoskeleton. Both conditions seem to be unfavourable when endothelium is challenged by shear stress. Obviously, aldosterone limits the compliance of the endothelium. In other words, a more deformable ('soft') endothelial cell could withstand collisions with the corpuscular elements of the blood better than a less elastic ('stiff') cell. It is tempting to speculate that under physiological conditions, i.e. when blood pressure is normal and/or aldosterone concentration is low, no problems occur. However, under pathophysiological conditions, i.e. when blood pressure is high and/or aldosterone concentration elevated, endothelial function could be hampered. This could finally lead to vascular damage observed in aldosteronism (Duprez *et al.* 2000).

Conflict of Interest

We declare that there are no conflicts of interest.

 doi: 10.1111/j.1748-1716.2006.01574.x

The study was supported by the Deutsche Forschungsgemeinschaft, SFB 629 A6, DFG Re 1284/2-1 and by EU grant Tips4cells. The technical support of Digital Instruments (VEECO, Mannheim) is gratefully acknowledged.

References

Duprez, D., De Buyzere, M., Rietzschel, E.R. & Clement, D.L. 2000. Aldosterone and vascular damage. *Curr Hypertens Rep* **2**, 327–334.

Garner, M.M. & Burg, M.B. 1994. Macromolecular crowding and confinement in cells exposed to hypertonicity. *Am J Physiol* **266**, C877–C892.

Gekle, M., Golenhofen, N., Oberleithner, H. & Silbernagl, S. 1996. Rapid activation of Na^+/H^+ exchange by aldosterone in renal epithelial cells requires Ca^{2+} and stimulation of a plasma membrane proton conductance. *Proc Natl Acad Sci USA* **93**, 10500–10504.

Goerge, T., Niemeyer, A., Rogge, P., Ossig, R., Oberleithner, H. & Schneider, S.W. 2002. Secretion pores in human endothelial cells during acute hypoxia. *J Membrane Biol* **187**, 203–211.

Golestaneh, N., Klein, C., Valamanesh, F., Suarez, G., Agarwal, M.K. & Mirshahi, M. 2001. Mineralocorticoid receptor-mediated signaling regulates the ion gated sodium channel in vascular endothelial cells and requires an intact cytoskeleton. *Biochem Bioph Res Co* **280**, 1300–1306.

Grossmann, C., Benesic, A., Krug, A.W., Freudinger, R., Mildenberger, S., Gassner, B. & Gekle, M. 2005. Human mineralocorticoid receptor expression renders cells responsive for nongenotropic aldosterone actions. *Mol Endocrinol* **19**, 1697–1710.

Henderson, R.M. & Oberleithner, H. 2000. Pushing, pulling, dragging, and vibrating renal epithelia by using atomic force microscopy. *Am J Physiol Renal Physiol* **278**, F689–F701.

Henderson, R.M., Schneider, S., Li, Q., Hornby, D., White, S.J. & Oberleithner, H. 1996. Imaging ROMK1 inwardly rectifying ATP-sensitive K+ channel protein using atomic force microscopy. *Proc Natl Acad Sci USA* **93**, 8756–8760.

Jaffe, E.A., Nachman, R.L., Becker, C.G. & Minick, C.R. 1973. Culture of human endothelial cells derived from umbilical veins. Identification by morphologic and immunologic criteria. *J Clin Invest* **52**, 2745–2756.

Kataoka, N., Iwaki, K., Hashimoto, K., Mochizuki, S., Ogasawara, Y., Sato, M., Tsujioka, K. & Kajiya, F. 2002. Measurements of endothelial cell-to-cell and cell-to-substrate gaps and micromechanical properties of endothelial cells during monocyte adhesion. *Proc Natl Acad Sci USA* **99**, 15638–15643.

Maguire, D., MacNamara, B., Cuffe, J.E., Winter, D., Doolan, C.M., Urbach, V., O'Sullivan, G.C. & Harvey, B.J. 1999. Rapid responses to aldosterone in human distal colon. *Steroids* **64**, 51–63.

Mathur, A.B., Collinsworth, A.M., Reichert, W.M., Kraus, W.E. & Truskey, G.A. 2001. Endothelial, cardiac muscle and skeletal muscle exhibit different viscous and elastic properties as determined by atomic force microscopy. *J Biomech* **34**, 1545–1553.

Mendelsohn, M.E. 2005. In hypertension, the kidney is not always the heart of the matter. *J Clin Invest* **115**, 840–844.

Oberleithner, H. 2004. Unorthodox sites and modes of aldosterone action. *News Physiol Sci* **19**, 51–54.

Oberleithner, H. 2005. Aldosterone makes human endothelium stiff and vulnerable. *Kidney Int* **67**, 1680–1682.

Oberleithner, H., Ludwig, T., Riethmuller, C., Hillebrand, U., Albermann, L., Schafer, C., Shahin, V. & Schillers, H. 2004. Human endothelium, target for aldosterone. *Hypertension* **43**, 952–956.

Oberleithner, H., Schneider, S.W., Albermann, L., Hillebrand, U., Ludwig, T., Riethmuller, C., Shahin, V., Schafer, C. & Schillers, H. 2003. Endothelial cell swelling by aldosterone. *J Membr Biol* **196**, 163–172.

Radmacher, M., Fritz, M., Kacher, C.M., Cleveland, J.P. & Hansma, P.K. 1996. Measuring the viscoelastic properties of human platelets with the atomic force microscope. *Biophys J* **70**, 556–567.

Schafer, C., Shahin, V., Albermann, L., Hug, M.J., Reinhardt, J., Schillers, H., Schneider, S.W. & Oberleithner, H. 2002. Aldosterone signaling pathway across the nuclear envelope. *Proc Natl Acad Sci USA* **99**, 7154–7159.

Schneider, M., Ulsenheimer, A., Christ, M. & Wehling, M. 1997. Nongenomic effects of aldosterone on intracellular calcium in porcine endothelial cells. *Am J Physiol* **272**, E616–E620.

Schneider, S.W., Matzke, R., Radmacher, M. & Oberleithner, H. 2004. Shape and volume of living aldosterone-sensitive cells imaged with the atomic force microscope. *Methods Mol Biol* **242**, 255–279.

Stier, C.T. Jr, Chander, P.N. & Rocha, R. 2002. Aldosterone as a mediator in cardiovascular injury. *Cardiol Rev* **10**, 97–107.

  doi: 10.1111/j.1748-1716.2006.01574.x