Kidney International, Vol. 67 (2005), pp. 1680–1682

Aldosterone makes human endothelium stiff and vulnerable

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Background. Aldosterone has long been known to control water and electrolyte balance by acting on mineralocorticoid receptors in kidney. However, recent studies identified these classic receptors also in the vascular system. Indeed, aldosterone alters structure and function of human endothelium. We applied nanotechniques to detect these changes.

Methods. Experiments were performed in cultured monolayers of human umbilical vein endothelial cells (HUVECs). Ten nanomoles per liter aldosterone or solvent (control) was added to the culture medium for 72 hours. Atomic force microscopy (AFM) was used as a nanotool that physically interacts with the cell surface. In the monolayer we measured cell volume, apical cell surface, and cell stiffness.

Results. Volume, apical surface area, and stiffness of individual cells increased in response to aldosterone by 18% ($P < 0.05$), 64% ($P < 0.001$), and 78% ($P < 0.01$), respectively. Imaging of the cell-to-cell contacts disclosed gaps in response to aldosterone. Furthermore, underneath the monolayer we detected an aldosterone-induced increase of protein deposition by 58% ($P < 0.001$).

Conclusion. Aldosterone remodels human endothelium in vitro. Cells increase in size and stiffness. Protein leakage through intercellular gaps could be caused by the increased apical membrane tension. The increase in cell rigidity could trigger endothelial dysfunction observed in hyperaldosteronism.

Aldosterone acts not only on epithelial cells of kidney and colon but also at nonepithelial sites in the brain, heart, and vasculature. A growing number of studies indicate that aldosterone plays a major role in the development of heart failure, myocardial fibrosis, and endothelial dysfunction [1]. By means of atomic force microscopy (AFM), a nanotechnique that allows to measure single cell volume independent of cell shape, apical endothelial cell surface, and membrane tension, we addressed the question of aldosterone-induced structural and functional remodeling of the endothelium.

METHODS

Culture of human umbilical venous endothelial cells (HUVECs) [2, 3] and cell imaging by AFM of fixed cells in fluid [4] has been described in detail. For measurements of cell stiffness [3, 5, 6] the AFM is used as a mechanical sensor. The AFM tip is pressed against the living (nonfixed) cell so that the membrane is indented. At the same time, the AFM cantilever that serves as a soft spring (spring constant 0.01 N/m) is distorted. Force distance curves quantify the force (pN) necessary to distort the membrane for a given distance (nm). In order to evaluate the nature and amount of material deposited underneath the monolayer cells were removed from the solid support by calcium chelation. Then, the solid support was scanned in fluid by AFM and the deposited material quantified as "mean surface roughness." This value indicates the mean height deviations of the deposited material in reference to the substratum.

RESULTS

HUVECs maintain natural shape and volume when fixed under physiologic conditions and scanned in liquid [3]. Images in Figure 1 are representative examples. They indicate a substantial increase in volume and apical membrane surface of aldosterone-treated cells. Consequently, the number of cells per defined area is reduced after hormone treatment. Table 1 shows the statistical analysis. According to the law of Laplace, the mechanical tension of a spherical structure correlates positively with the radius. Since cells can be treated as segments of a sphere, we measured the force necessary to indent the cell membrane by a given distance. Indeed, we detected a 78% ($P < 0.001$) increase of membrane tension (Table 1).

Close inspection of the HUVEC monolayer in liquid disclosed gap formation between cells of the aldosterone-treated monolayers (Fig. 1). The gaps were found inhomogenously distributed in the monolayer. We hypothesized that such structures could serve as paracellular pathways for macromolecules traveling from the apical to the basolateral side of the endothelium. Therefore, in another series of experiments, we removed the endothelial cells by exposure of the monolayer to the calcium-chelating agent ethylenediaminetetraacetic acid (EDTA). Then we scanned the coverslips and found material deposited underneath the monolayer. Obviously, macromolecules accumulated there after aldosterone

Key words: atomic force microscopy, cell stiffness, endothelial compliance.

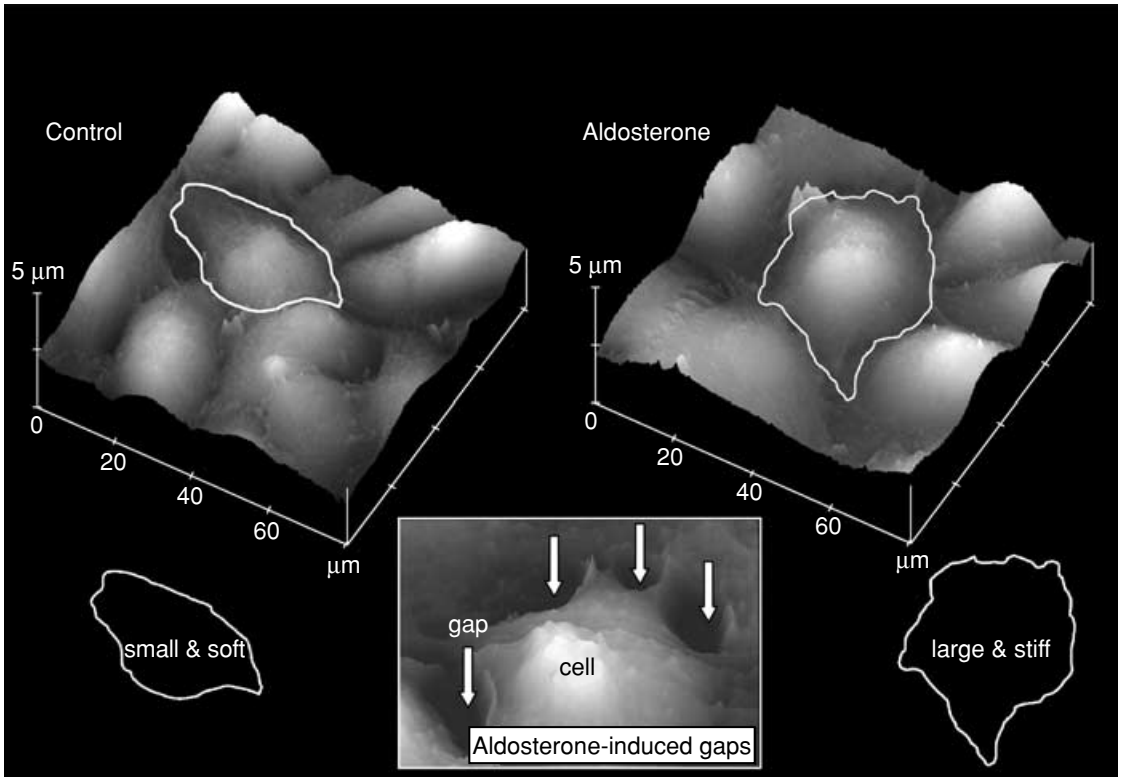

Fig. 1. Atomic force microscopy (AFM) images of human umbilical venous endothelial cells (HUVECs) monolayers. Cells were exposed to either the solvent (control) or 10 nmol/L aldosterone for 72 hours. Outlines indicate single cells. Cell size and stiffness increase with aldosterone exposure. Intercellular gaps occur in response to the hormone.

Table 1. Remodeling of human endothelial cells in response to aldosterone

Parameter	Control	Aldosterone	Increase%
Single cell volume *fl*	1652 ± 63 ($N = 10$)	1996 ± 161 ($N = 10$)	18 ($P < 0.01$)
Apical cell surface μm^2	1238 ± 52 ($N = 10$)	2035 ± 170 ($N = 10$)	64 ($P < 0.001$)
Cell stiffness *pN/nm*	6 ± 0.7 ($N = 10$)	10 ± 1.2 ($N = 14$)	78 ($P < 0.01$)
Mean roughness *nm*	1.4 ± 0.08 ($N = 9$)	2.1 ± 0.07 ($N = 9$)	56 ($P < 0.001$)

Aldosterone exposure was 72 hours. Mean roughness is a measure for the amount of proteins deposited underneath the endothelial cell monolayer (mean values ± SEM) (N = number of experiments). All data were obtained with atomic force microscopy (AFM). P values indicate the level of significance comparing mean data of the control and aldosterone experiments.

treatment (expressed as mean surface roughness in Table 1). Short exposure to trypsin decreased the amount of this material indicating that the detected matter consisted of proteins.

DISCUSSION

Renal epithelium and vascular endothelium share certain properties: They both express mineralocorticoid receptors [7, 8] and epithelial sodium channels [9]. Moreover, the endothelium responds to the epithelial sodium channel blocker amiloride [10]. However, aldosterone should not be considered to be just a hormone in charge of the recruitment of sodium channels. Its proliferative action on cells, particular on renal epithelium, has been recently described [11]. Thus, numerous ion channels and transporters are expected to be expressed in response to aldosterone. This could explain the 18% increase of endothelial volume [10] and the 64% increase of the apical endothelial membrane surface. Furthermore, aldosterone exposure facilitates intercellular gap formation. Due to the gap size in the range of micrometers they could serve as irregular diffusion pathways for large particles. This should alter the selectivity of the endothelial filter. Indeed, large particles, most likely proteins/protein complexes, accumulate more readily underneath the monolayer. This is indicated by the dramatic increase in "roughness" measurements of the cell substratum after removal of the cells. It is tempting to assume that aldosterone-induced gaps allow large proteins and even supramolecular structures to pass the endothelium in an irregular "nonselective" manner.

Another observation is the increase in endothelial cell stiffness with aldosterone treatment. This could be the result of the increased apical membrane tension in

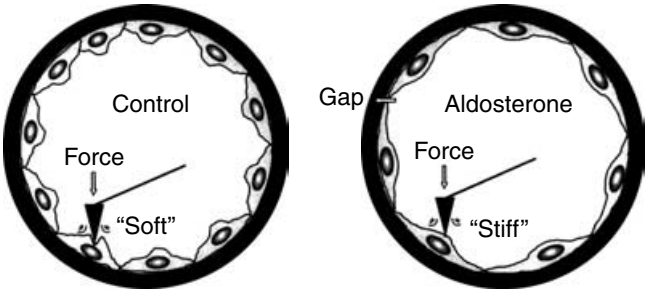

Fig. 2. Schematic on the structural and functional changes in endothelium induced by aldosterone. Cells grow in size and surface while the number of cells per area of endothelium diminishes. Cell flattening causes increased membrane tension and intercellular gap formation. Cells get stiff and unselective endothelial leaks occur.

hormone treated cells (Fig. 2). An increase in cell stiffness is expected according to the law of Laplace [12]. Membrane tension should increase with increasing cell diameter. Besides this biophysical explanation aldosterone-induced stiffness of endothelial cells could be caused also by the modulation of NADH/NADPH-dependent oxidases [13] and/or the nitric oxide synthase pathway [14]. Presumably, aldosterone-triggered formation of superoxide anion, a potent scavenger of nitric oxide, could stiffen endothelial cells by yet unknown molecular mechanisms.

It is tempting to speculate that during aldosterone exposure a small percentage of cell-to-cell contacts cannot withstand the increased mechanical forces (i.e., membrane tension) and, therefore, develop gaps. Such a nonselective pathway could contribute to endothelial dysfunction observed in hyperaldosteronism [15].

ACKNOWLEDGMENTS

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

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