Unorthodox Sites and Modes of Aldosterone Action

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Aldosterone controls electrolyte balance by acting on the renal epithelium. However, there is strong evidence that vascular endothelium is another target for mineralocorticoids. Endothelial cells gain sensitivity to diuretics when exposed to aldosterone. Atomic force microscopy detects such phenomena. It is speculated that endothelium and kidney join forces in the regulation of body fluids.

When you read about aldosterone action in a student’s textbook of medical physiology, you usually get the following information: the mineralocorticoid hormone aldosterone is synthesized in the suprarenal gland and, after release into the bloodstream, acts on target cells of the renal collecting duct. There aldosterone binds to intracellular mineralocorticoid receptors, and after translocation into the cell nucleus, transcription is initiated. Aldosterone-induced mRNA is exported and then translated at the ribosomes. After some delay, aldosterone-induced proteins appear in the cell and finally change the function of the nephron. Sodium is retained in the body and potassium is excreted, which is the classic response of aldosterone in kidney.

There is nothing wrong with this summary. However, recent developments in this field indicate that the story is still incomplete and that other sites and modes of action of this hormone should be considered.

Time course of aldosterone action

Aldosterone controls body fluids and electrolytes. Whenever blood pressure falls below a certain threshold, the renin-angiotensin-aldosterone system (RAAS) is activated and more salt and water are reabsorbed in the kidney. Although the physiological response only becomes effective after hours, cellular mechanisms in the distal nephron are more or less immediate.

One of the first signals elicited by the hormone is a transient increase of intracellular calcium. This response occurs within seconds and lasts for minutes. Interestingly, the change in calcium can be observed not only in renal cells (4) but also in other tissues (3). The physiological relevance of this calcium increase could, among other mechanisms, lie in a so far ill-defined interaction of the mineralocorticoid receptors with the hormone or in a calcium-dependent translocation of the activated receptor through the nuclear pore complexes. By a combination of atomic force microscopy (AFM) with electrical techniques applied in Xenopus laevis oocytes, we found that aldosterone leads within minutes to receptor docking at the nuclear pore complexes followed by subsequent receptor translocation (14). Inhibition of the initial intracellular calcium peak blocks this first step of the genomic response (9).

Another fast cellular response of aldosterone, detected more than 16 years ago in amphibian kidney cells (11), is the activation of plasma membrane Na+/H+ exchange. Whether this widely observed aldosterone-induced response is a prerequisite for the later transcriptional processes or merely an epiphenomenon associated with the metabolic alterations in the target cell is still unknown.

About 20 min after stimulation, the first mRNA transcripts exit the nucleus as directly shown by AFM (Fig. 1). mRNA appears as so-called plugs in the central channels of nuclear pore complexes (14). Studies from other laboratories showed that one of the first proteins triggered by the hormone is the serum glucocorticoid kinase (sgk), an enzyme that regulates the activity of plasma membrane Na+ channels (12). Indeed, we recently identified the plugs by nested PCR methods as possibly being sgk transcripts (unpublished observations in the author’s laboratory).

In conclusion, the determination of the response time of aldosterone depends on the experimental approach. If blood pressure or extracellular volume is the measured parameter, the response time is in the range of hours. If mRNA appearance is the measured parameter, then the response time is <1 min. Finally, if intracellular electrolytes are measured, aldosterone action is virtually immediate.

Sites of aldosterone synthesis and action

Aldosterone synthesis and action occur at various locations in the human body. There is strong evidence that, besides the suprarenal glands, aldosterone is also synthesized in heart (16) and blood vessels (18). At these locations, it is regulated by similar mechanisms to the RAAS. Because aldosterone acts on cardiomyocytes, cardiac fibroblasts, and endothelial cells, it plays a major role in the development of heart failure, myocardial fibrosis, and endothelial dysfunction (17). Moreover, there is much interest in the possibility of using aldosterone receptor blockade in patients to diminish pathological effects that can be produced by this hormone (13, 20).

A study applying AFM on living aortic endothelial cells showed transient cell swelling that occurred over minutes and that was prevented by the diuretic amiloride (15). Although the underlying mechanism and its physiological relevance...
were still unclear at the time, attention was thrown on data suggesting that endothelial cells not only synthesize aldosterone (18) but also express mineralocorticoid receptors (8) and the epithelial sodium channel (6).

Recent experiments in primary cultures of human endothelial cells (HUVEC) confirmed that they express the mineralocorticoid receptor (10). It was tempting to speculate that, as in target cells of the kidney, aldosterone should alter cellular ion and water transport and thus change cell volume measured by AFM. This technical approach allows simultaneous detection of any changes in cell volume and in cell shape (Fig. 2).

Indeed, we found a genomic effect of aldosterone in this “unorthodox” cell model (10). HUVEC swell in response to the hormone, a phenomenon inhibited by the mineralocorticoid receptor antagonist spironolactone or by the sodium channel blocker amiloride.

Mechanism of aldosterone-induced endothelial cell swelling

We speculate that the mode of aldosterone action in endothelial cells could be similar compared with renal collecting duct cells. We envision the following scenario. Activation of sodium channels causes cells to electrically depolarize due to increased sodium influx. Although sodium is pumped out of the cell by the Na⁺-K⁺-ATPase, which is located in the...
basolateral membrane, Cl\(^-\) accumulates in the cell due to electrochemical driving forces set by the altered cell membrane potential. This causes intracellular retention of water and thus cell swelling (Fig. 3).

The amiloride response could be explained along the same lines. It is evident that only cells with a significant sodium permeability, based on epithelial sodium channel activity, can respond to amiloride. Such an increased sodium permeability exists in HUVEC only in the presence of aldosterone. Under these conditions, application of amiloride blocks the apical sodium channels and thus hyperpolarizes the cell membrane. Electrochemical driving forces lead to Cl\(^-\) efflux. Concomitantly, water leaves the cell and shrinkage occurs. This could explain the observation that HUVEC volume is insensitive to amiloride before aldosterone exposure but becomes sensitive to the diuretic after aldosterone treatment. Nevertheless, more data on aldosterone action in endothelia are necessary to place this hypothesis on firm grounds.

Endothelial cell swelling is a phenomenon observed in ischemia and lactacidosis (1). It is based on the activation of the plasma membrane Na\(^+\)/H\(^+\) exchanger inhibited by amiloride analogs such as ethylisopropylamiloride. Although aldosterone can exert rapid responses through activation of Na\(^+\)/H\(^+\) exchange (4, 7, 11, 19), this is not the mechanism underlying aldosterone-induced HUVEC swelling. Low amiloride concentrations (1 μM) do not affect the activity of the Na\(^+\)/H\(^+\) exchanger to a significant extent but block epithelial sodium channels. Furthermore, cariporide, a specific Na\(^+\)/H\(^+\) exchange blocker, turned out to be ineffective when applied instead of amiloride (10). This indicates that cell swelling is associated with increased Na\(^+\)/H\(^+\) exchange activity only if cells are challenged by intracellular acidosis. This is certainly not the case after aldosterone treatment. Rather, intracellular alkalosis is expected to occur, due to the favorable inwardly directed electrochemical driving forces for HCO\(_3\)\(^-\), induced by aldosterone-mediated cell depolarization.

Potential clinical relevance of unorthodox aldosterone action

Hypokalemic alkalosis and hypertension characterize the syndrome of primary aldosteronism (20). The prevalence of primary aldosteronism in patients with hypertension is in the range of 5–13% (20). Hypokalemia and hypertension have been attributed to the aldosterone-induced altered function of the kidney. Aldosterone activates sodium and potassium channels in principal cells of distal tubule and collecting duct, leading to sodium reabsorption and potassium secretion (5). In parallel, acid equivalents are secreted in the distal nephron.

However, aldosterone acts not only on epithelial cells of kidney and colon but also at nonepithelial sites in brain, heart, and vasculature (17). We want to emphasize the potential role of endothelial cells in the physiology and pathophysiology of aldosterone action. Since endothelial cells and renal epithelial cells obviously respond to aldosterone in similar ways, endothelial cells should help to retain sodium in the organism and to release potassium. Again, this hypothesis needs further experimental testing.

At physiological aldosterone concentrations, endothelial cells support the kidney by rapidly adjusting extracellular volume and plasma potassium concentration. In aldosteronism, endothelial cells can release large amounts of potassium into the blood because of cell depolarization caused by the aldosterone-activated sodium channels. Since the total volume of endothelial cells in the adult human organism is in the same range as total blood volume (~5 l) and since total endothelial cell surface is extremely large (~1,000 m\(^2\)), there is a huge potassium buffer available that can be shifted rapidly.

Through mineralocorticoid receptors in endothelium and renal epithelium, the two structures could be functionally linked. Whenever the organism is threatened by a potassium overload, aldosterone activates potassium secretory mechanisms in distal nephron while potassium is released from the
endothelial cells into the blood. Thus aldosteronism will deplete the organism of potassium and finally cause hypokalemia.

Diuretics operate at unorthodox sites

This view opens new perspectives concerning treatment of diseases that involve high aldosterone concentrations. Spironolactone and analogs have recently turned out to be extremely useful in the treatment of cardiovascular dysfunctions (13, 20). This has been mainly attributed to the action of the mineralocorticoid receptor antagonists in cardiomyocytes and cardiac fibroblasts.

Amiloride is a potassium-sparing diuretic that clearly acts on sodium channels of kidney tubules. At usual daily dosages of 5–10 mg, amiloride reaches micromolar concentrations in renal collecting ducts, due to renal concentrating mechanisms (2). Such concentrations are high enough to block the luminal sodium channels. In peripheral blood, concentrations are 10–100 times lower, possibly too low to affect the sodium channels of the endothelium during amiloride treatment. Nevertheless, a single amiloride injection could at least transiently hyperpolarize endothelial cells of a patient with aldosteronism and thus could lead to transient shifts of potassium from blood to endothelium.

Unorthodox outlook

We postulate a significant role of the endothelium in salt and fluid homeostasis regulated by aldosterone. As a consequence, the endothelial cells may play a bigger role in the regulation of blood pressure. There could be indeed a functional linkage between endothelium and kidney, more than as yet anticipated.

I apologize for significant work not cited in this review due to space limitations.

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References