MINIREVIEW

Extrarenal receptor-effector-mechanisms for aldosterone: The sequence of effects on the cellular electrolyte transport in human lymphocytes and their implications for disorders of the water and electrolyte balances

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Abstract. High affinity aldosterone binding sites have not only been described in the classic target tissues such as the renal tubules, but also in non-classic target tissues such as the hippocampus, mammary gland, endothelial cells and, recently, human mononuclear leukocytes. An in vitro effect of aldosterone on intracellular sodium, potassium and calcium concentrations and cell volume was shown in human mononuclear leukocytes. In the absence of aldosterone, the intracellular Na⁺, K⁺ and Ca²⁺ concentrations and the cell volume decreased significantly, but remained constant when aldosterone (1.4 nmol/l) was added to the incubation medium. These effects of aldosterone were blocked by the aldosterone antagonist canrenone (140 nmol/l). The sodium/proton exchanger of the cell membrane could be identified as the primary target of the aldosterone action, possibly non-genomically mediated through membrane receptors. The clinical significance of this model was underlined by the demonstration of absent or a decreased number of mineralocorticoid receptors and the lack of electrolyte response to aldosterone in human mononuclear leukocytes of patients with pseudohypoaldosteronism and aldosteronism. Additionally, an abnormal effector mechanism could be demonstrated in human mononuclear leukocytes from essential hypertensives. These studies are the first to demonstrate the significance of extrarenal, nonepithelial mineralocorticoid receptors and the related effector mechanism in different disorders of the water and electrolyte balance in man.

1. Extrarenal mineralocorticoid receptors and effects on electrolyte transport

Extrarenal mineralocorticoid receptors
Aldosterone regulates the transport of Na⁺ and K⁺ in target tissues at the level of the plasma membrane. This effector mechanism involves binding of aldosterone to type I receptors with subsequent synthesis of a specific protein. High-affinity aldosterone binding sites have not only been described in classic target tissues such as kidney tubules, but also in non-classic target tissues such as the hippocampus, mammary gland, heart, spleen, and pituitary gland. Data on mineralocorticoid receptors in HML based on molecular biology methods are not yet available. Therefore, our knowledge about mineralocorticoid receptors in HML still depends on classic binding experiments solely.

Mechanisms of extrarenal mineralocorticoid effects
Long-term effects of mineralocorticoids on the blood pressure and the electrolyte balance are commonly understood as a result of renal effects mainly located at the distal tubule. Though yet unknown in detail, this effector mechanism seems to involve the binding of aldosterone to a cytoplasmic receptor protein inducing the nuclear production of mRNA. From this mRNA a specific protein is translated which is thought to create "new" sodium channels in the cell membrane.

This protein facilitates the passive entrance of sodium into the cells and the intracellular concent...
tration of sodium is increased. As a second step, the increased intracellular sodium concentration activates and/or induces the production of Na-K-ATPase (10). Owing to the polarity of the membrane distribution of Na-K-ATPase in tubular cells with a high concentration at the basal membrane, a tran-
secular transport of sodium and potassium is typical for these cells (11). The renal component of mineralocorticoid-induced hypertension thus mainly reflects the sodium and water retention and the consequent increase in the cardiac output. However, several observations about deoxycorticosterone-acetate (DOCA)-induced hypertension do not support this mechanism as the only cause of mineralocorticoid-induced hypertension.

In DOCA-treated young pigs arterial hypertension was found to be due to a marked elevation of peripheral resistance and increases in the extracel-
ular space and cardiac output (12). Beta-blockade in DOCA-treated dogs resulted in a normalization of the cardiac output but failed to correct the ar-
terial hypertension (13). A similar observation was reported for dogs after 16 days of parenteral aldosterone medication (14). In these animals the increase in blood pressure was not paralleled by increases in cardiac output and was thus related to increases in peripheral resistance.

In even older studies severe doubts about the renal involvement in the development of DOCA-
induced hypertension were raised: in nephrecto-
mized dogs and rats arterial hypertension could be induced by DOCA and salt per se (15). Of course, these effects are potentiated by renal salt and water retention.

These data show that major circulatory effects of mineralocorticoids are of extra-renal origin. In the past few years several studies dealing with mine-
ralocorticoid effects on extrarenal tissues were published.

Jones (16) and Jones & Hart (17) demonstrated an increase in the membrane permeability for monovalent cations in smooth muscle cells from DOCA-hypertensive rats. Using a different technique Friedman & Friedman (18) also found an increased permeability for sodium, potassium and lithium in smooth muscle cells from DOCA-hypertensive rats compared with cells from normal animals. Kornel et al. (19) demonstrated a stimulated influx of sodium into smooth muscle cells from the aorta of DOCA-treated rats and correlated this effect with mineralocorticoid binding to cytosolic receptors.

Moura & Worcel (20) reported an increase in transmembranal sodium flux in the tail artery of adrenalectomized rats in which the efflux of $^{22}$Na was measured after injection of aldosterone (10 μg/kg). The ouabain-independent efflux of $^{22}$Na was increased as early as 10-20 min after aldoste-
rone-injection, whereas changes of the ouabain-independent sodium efflux started after 1 h. The effects persisted up to 5 h after exposure to aldosterone. The inhibitor of transcription actinomycin D did not alter the rapid responses to aldosterone. Like the sequence of mineralocorticoid effects in kidney cells, the results on smooth muscle cells are consistent with the hypothesis that aldosterone can increase both entry and exit of Na$^+$, primarily by increasing plasma membrane permeability, and secondarily by increasing the Na-K-ATPase activity. This activation of Na-K-ATPase does not only lead to an increase in sodium extrusion as mentioned above, but also to an increase in intracellular potassium since Na-K-ATPase extrudes sodium in exchange for potassium. The resultant of both the primary increase in sodium influx and secondary activation of Na-K-ATPase by chronic administration of mineralocorticoids was experimentally demonstrated by Webb (21) for smooth muscle cells in DOCA-hypertensive pigs. As an indirect evidence for its vascular effects, a sensitization of the vascular reactivity to constrictory stimuli such as norepinephrine, vasopressin, angiotensin II and serotonin was shown (22,23) which preceded the rise of blood pressure by mineralocorticoids.

Another extrarenal effect of mineralocorticoids with possible relevance for its hemodynamic action involves the central nervous system. The increased sensitivity of the vascular reactivity to constrictory stimuli as mentioned above could be attributed to an activation of the sympathetic nerve system (24). On the other hand, a selective destruction of the AV3V region in the rat brain blocks the development of a DOCA-induced hypertension (25,26). Though not known in detail, the mechanism of this intervention seems to involve the central angioten-
sin-pressor-mechanism and changes in the secre-
tion of neurohumoral factors such as vasopressin and the endogenous Na-K-ATPase-inhibitor.

2. In vitro-studies on human mononuclear leukocytes

Demonstration of mineralocorticoid receptors

The investigations mentioned above regarding extrarenal mineralocorticoid effects were also per-
formed to find an easily accessible cell model for these effects since the classic target organ kidney is not viable for in vitro studies of isolated cells. It, therefore, was our aim to clarify the mineralocorticoid receptor status of human blood cells.

Specific [3H]aldosterone binding to human mononuclear leukocytes separated from peripheral blood by Percoll® was measured in the presence of a 5000-fold excess of the synthetic, "pure" glucocorticoid RU 26988. This compound restricts the binding of aldosterone to mineralocorticoid receptors and thus allows the selective study of these receptors. The affinity constant of aldosterone binding to HML mineralocorticoid receptors was in the physiological range at the plasma aldosterone concentration of about 1.5 nmol/l, the number of receptors/cell was 200-400 (4). No aldosterone binding to platelets and granulocytes was observed. The specific aldosterone antagonist potassium canrenoate competitively inhibited aldosterone binding. The affinity of cortisol for the receptor was 1/50 of that of aldosterone.

Mineralocorticoid effects on the cellular electrolyte and water metabolism

Demonstration of specific mineralocorticoid binding to HML did not prove the physiological function of the binding site as a "receptor" with relevance for the cellular electrolyte and water metabolism. To address this question, HML were separated from blood by a Percoll gradient and intracellular Na+ and K+ were determined by flame photometry before and after incubation for 1 h at 37°C with or without aldosterone. The intracellular Na+ and K+ concentrations after separation of HML were 17±5 and 59±18 nmol/kg wet cells (mean ± SD, N=6), respectively. In the absence of aldosterone the intracellular Na+ concentration decreased to 12±4, whereas the intracellular Na+ concentration remained constant at 18±8 nmol/kg wet cells when aldosterone (1.4 nmol/l) was added to the incubation medium. In parallel, the intracellular K+ concentration decreased in the absence of aldosterone, but remained constant when aldosterone was added. The effect of aldosterone on the intracellular Na+ and K+ concentrations of HML was near maximal at 0.14 nmol/l and it was blocked by the aldosterone antagonist canrenoene (140 nmol/l). Cortisol at a physiological concentration (40 nmol/l) did not alter the intracellular Na+ and K+ concentrations in these cells (27). The results suggested that aldosterone binding to specific receptors in human mononuclear leukocytes significantly contributes to the regulation of monovalent cation levels in these cells.

Free intracellular calcium Ca2+i was measured by Quin2 fluorescence after incubation of HML for 1 h at 37°C in RPMI-1640 medium with or without aldosterone. After incubation without aldosterone Ca2+i in HML was 118±27 nmol/l. After incubation with aldosterone Ca2+i was increased to 139±38 nmol/l (p<0.05, N=11, mean ± SD, 28). By use of Fura2, Ca2+i was 56±15 nmol/l in fresh HML (mean ± SD, N=26). After incubation without aldosterone Ca2+i significantly fell to 51±14 nmol/l (p<0.001). After incubation with 1.4 nmol/l aldosterone Ca2+i remained constant at 57±11 nmol/l (p<0.0005). These effects were antagonized by amiloride, actinomycin D and canrenoene. Cortisol was a partial agonist.

Since the concordant changes of sodium and potassium were expected to be accompanied by water and volume shifts, HML volume was determined from the measurement of cell diameter and planimetry of the cell area in photographs. Cell volume decreased by about 15% when cells were incubated in RPMI-medium without aldosterone for 1 h at 37°C. This decrease was absent when 1.4 nmol/l aldosterone was added to the incubation medium; the effect was half maximal at a concentration between 0.07 and 0.14 nmol/l; 140 nmol/l canrenoene antagonized the action of aldosterone. Cortisol was ineffective (29).

The results indicate concordant changes of intracellular sodium, potassium, calcium and cell volume if studied under the same conditions. These data are the first to demonstrate that aldosterone is a major physiological determinant for the regulation of lymphocytic volume in isotonic media. There is a precedent for a unidirectional effect of mineralocorticoids on Na+ and K+ in studies of DOCA-hypertensive rats in which an increase in both intralymphocytic Na+ and K+ was observed in response to DOCA (8). In other situations a parallel change of Na+ and K+ in HML was seen after an increase in intracellular sodium with subsequent stimulation of Na-K-ATPase.

If exposed to hypertonic media, HML shrink rapidly and then regain volume (regulatory volume increment, RVI) mainly by the activation of the sodium-proton exchanger of the cell membrane and a subsequent sodium gain of the cell, which is inhibited by amiloride (30). This cation transport is coupled with a hydrogencarbonate-chloride ex-
change to balance the intracellular alkalization, thus sodium chloride enters the cell during RVI (31-33). This gain of sodium is paralleled by an increase in intracellular potassium by an activation of Na-K-ATPase (30). For the RVI, the concordant increase in both intracellular sodium and potassium has been observed in consequence of a primary increase in sodium influx and a secondary activation of Na-K-ATPase. The same holds true for the RVI in amphibious red blood cells which also involves an activation of both the sodium-proton exchanger and Na-K-ATPase, resulting in a concordant increase in both sodium and potassium (34,35). Activation of both the sodium-proton exchanger and the Cl⁻-HCO₃ exchanger has also been shown for epithelial cells of the necturus gall bladder after osmotic shrinkage (36) and for the basolateral membrane of the Henle loop in the mouse (37).

In Fig. 1 the polar kidney tubular cell and an apolar cell are compared with regard to the electrolyte transport processes related to mineralocorticoid action. The main difference between the two cell types results from the polarity of the Na-K-ATPase distribution in polar cells which causes electrolyte transport across epithelia. In apolar cells, mineralocorticoid effects may be monitored only by the resulting changes in transmembranal electrolyte fluxes and intracellular electrolyte concentrations.

Free intracellular calcium is commonly considered as second messenger for the triggering and regulation of the contractile force which may be influenced by intracellular sodium via a Na/Ca exchanger in the plasma membrane (38). There is an ongoing debate about the existence of this system in HML. Depolarization, removal of extracellular sodium or sodium loading of HML did not alter the Ca²⁺i as would be expected if a Na/Ca exchanger was active (39-41). The only study by which the existence of this transporter is indicated was done in membrane vesicles from lymphocytes (42).

Fig. 1.

a. In the polar cells, the concentration of Na-K-ATPase at the basilar membrane leads to a transcellular transport, whereas in the apolar cells the mineralocorticoid action is measurable only through changes of the intracellular electrolyte concentrations and transmembranous fluxes.

b. Comparison of the principle effects of aldosterone on the cellular sodium transport in a polar renal tubule cell (left) and an apolar, non-epithelial cell, e.g. a lymphocyte (right). In both cases, the sodium influx into the cells is increased primarily; the subsequent activation of Na-K-ATPase results in an active sodium extrusion thus maintaining the cellular sodium homeostasis and, in the polar cell, in transepithelial ion transport.
Na/Ca exchange was detectable in this preparation. The criticism concerning the methods of this study includes the possibility of admixture of organelles like mitochondria in the membrane preparation or other blood cells. In addition, the affinity constant for calcium of 61 μmol/l was too high for a physiologically active system at a normal Ca²⁺ of about 0.1 μmol/l. On the other hand, the results from our study support the existence of a Na/Ca exchanger. Also in vivo studies showed an increase in free intracellular calcium in human lymphocytes after chronic application of mineralocorticoids (43).

The sequence of mineralocorticoid effects on HML electrolytes and volume is summarized in Fig. 2. To a great extent, this cascade of single steps is analogous with the mineralocorticoid effects in renal tubular cells as far as known. As mentioned above the specific properties of polar epithelial cells, such as transepithelial transport, are mainly related to the polar distribution of membrane enzymes (Na-K-ATPase), whereas the first steps of the mineralocorticoid effector cascade appear essentially to be identical in the two types of cells.

The data summarized so far do not address the question of the primary target for the mineralocorticoid action at the level of the cell membrane. In early studies, the induction of an apical increase in the sodium conductance of the tubular cell by a hypothetical "permease" was assumed. Electrophysiological methods demonstrated a short-circuit current which was inhibited by amiloride (10,44). The increased entrance of sodium into the renal cells resulted in a rise of the intracellular sodium concentration (45-48).

In a recent paper by Oberleithner et al. (49) early changes in the activity of the sodium-proton exchanger in the distal tubule of the toad kidney were observed after aldosterone, leading to alkalinization of the cytoplasm and an increase in potassium excretion into the lumen. The latency of this effect

![Diagram](https://via.placeholder.com/150)

**Fig. 2.**

The steps of mineralocorticoid effects on the cellular electrolyte and volume: the primary increase in the intracellular sodium concentration by a stimulation of the sodium-proton exchanger or the induction of sodium channels (a) activates Na-K-ATPase and results in a concordant increase in the intracellular potassium concentration (b). This parallel increase in sodium and potassium is accompanied by transmembrane shifts of water and thus changes of cell volume (c). Via the Na/Ca exchanger the free intracellular calcium is also increased (d). These effects were found in human mononuclear leukocytes and are discussed in the text.
The steps in the cascade of mineralocorticoid effects are shown in an apolar cell and classified as fast and late responses. The scheme both includes the possibility of direct membrane effects of mineralocorticoids and effects mediated by the nucleus.

is only 20 min. The authors point out that trans-
epithelial processes may have a longer latency period which could be related to secondary, electrogeneric processes. These changes could be detected only by electrophysiological means. The activation of the electroneutral sodium-proton exchanger as a possible primary target of the mineralocorticoid action is likely to be missed by these methods.

We therefore investigated the effects of aldoste-
rone on the sodium-proton exchanger in HML. By measuring the amiloride-sensitive swelling of HML in isotonic sodium propionate medium we found this transporter to be significantly stimulated, by 20–30%, as early as 3 min after incubation with the steroid. This action of aldosterone was highly sig-
ificant up to 30 min at concentrations above 0.1
nmol/l. It could be antagonized by amiloride cor-
tisol was a partial agonist (50).

Thus, the effects of aldosterone on electrolytes and cell volume in HML appear to be comparable to those initiated by the regulatory volume incre-
ment: aldosterone either directly or indirectly (via the nucleus) stimulates the sodium/proton ex-
changer increasing the intracellular sodium con-
centration. Thereby, Na-K-ATPase is activated and potassium exchanged for sodium. For osmotic reasons, the net increase in both cations and chloride (via the hydrogen carbonate/chloride exchanger) results in a swelling of the cells. The in-
crease in the intracellular cation concentrations is partially balanced by this. Free intracellular cal-
cium is raised by the increased intracellular sodium via the sodium-calcium exchanger. It is likely that these rapid effects of aldosterone on the sodium-
proton exchanger are mediated non-genomically by receptors different from the classic intracellular type I receptors which induce genomic responses. The sequence of mineralocorticoid effects is sum-
marized and classified as fast and late responses in Fig. 3.

Mineralocorticoid receptors and electrolyte effects in human mononuclear leukocytes from patients with disorders of the water and electrolyte balance

The clinical significance of mineralocorticoid re-
ceptors in HML was underlined by the demonstra-
tion of absent or decreased number of mineralo-
corticoid receptors in patients with pseudohypoaldosteronism (51). This disease is characterized by renal salt loss, leading to hyponatremia and hyperkalemia despite elevated levels of aldosterone and plasma renin activity. High doses of aldosterone remain ineffective, whereas sodium supplementation is able to correct the electrolyte abnormalities. However, while hormonal abnormalities and the receptor deficiency persist, the salt-wasting symptoms usually disappear within the first years of life (51). It has been concluded that the absent or decreased number of mineralocorticoid receptors in HML of patients with pseudohypoaldosteronism reflects a generalized defect which is also present in the renal tubules and thus explains the salt wasting in these patients. To study further the significance of mineralocorticoid receptors and the related effector mechanism for the sodium homeostasis in patients with pseudohypoaldosteronism, intracellular sodium and potassium in HML were measured after incubation with or without aldosterone. Obtained at the level of the effector mechanism, the results are in good agreement with the finding of a lack or decreased number of mineralocorticoid receptors in patients with pseudohypoaldosteronism. In HML of three patients with pseudohypoaldosteronism at an age of 6 months, 3 and 9 years the loss of intracellular sodium and potassium during incubation in a protein-free medium was not prevented by aldosterone as seen in normal children and adults (52). In addition, in the families of patients with pseudohypoaldosteronism a decreased number of mineralocorticoid receptors and a defective effector mechanism were demonstrated in one of the parents of each index case (53).

For patients with primary and secondary aldosteronism a reduced number of mineralocorticoid receptors has been shown on HML (54,55), thus indicating a down-regulation in response to chronically elevated serum levels of aldosterone. It was hypothesized that this down-regulation contributes to the escape not only of HML, but also of renal tubular cells from mineralocorticoid effects which blunts the development of more severe sodium retention and hypokalemia in these patients.

To study further the physiological implications of these findings at the cellular level, the mineralocorticoid effector mechanism was investigated in HML of 6 patients with primary aldosteronism. Except for one patient with elevated intracellular electrolytes, sodium and potassium in non-incubated mononuclear leukocytes of patients with aldosteronism were within the range for normals. For the patients no significant change of intracellular sodium or potassium was observed during incubation with or without aldosterone (1.4 nmol/l), whereas in normals the loss of sodium and potassium during incubation without aldosterone was prevented by 1.4 nmol/l aldosterone (56). This insensitivity to aldosterone indicates that intracellular electrolytes in mononuclear leukocytes of patients with primary aldosteronism are kept within normal ranges by mechanisms independent of mineralocorticoids and may represent the cellular correlate of the renal "escape" phenomenon in aldosteronism.

The HML model was also investigated in 13 patients with essential hypertension. In only 4 patients, sodium in non-incubated HML was elevated compared with the range for normals. A decrease of intracellular sodium or potassium occurred during incubation without aldosterone (57). The addition of 1.4 nmol/l aldosterone did not prevent this loss of electrolytes as observed in normals. Plasma renin and aldosterone were not correlated with the electrolyte response and were within the normal limits. The numbers of mineralocorticoid receptors/cell were within or close to the normal range. These findings indicate that in patients with essential hypertension, the intracellular electrolytes in HML are independent of the mineralocorticoid receptor-mediated effector mechanism by which intracellular sodium and potassium are elevated in vivo and in vitro. The most attractive explanation of this would be a counter-regulatory impairment of the electrolyte-elevating mineralocorticoid effector mechanism. It could be speculated that this impairment would blunt the increased sodium influx and intracellular sodium concentration as a result of a genuine membrane defect in essential hypertension (58).

3. Perspective

The data presented demonstrate the relation between the cellular mineralocorticoid receptor/effector axis and various disturbances of the water and electrolyte balance in man. The cells investigated are easily accessible and their handling does not require extensive prerequisites. The significance of the model is underlined by its simple applicability to clinical situations with limited or
lacking access to other cells for in vitro studies. The model seems to be a promising tool in the diagnosis of unrecognized and hereditary disorders of the water and electrolyte balance in man. Additionally, the impact of therapeutic regimens may be monitored by the model. The relatively large scattering of individual data means a restriction of reliability at the present state of the art, but improvements of the techniques employed, especially regarding the volume-related experiments, are currently developed.

Another promising question to be addressed in the future deals with the functional effects of mineralocorticoids on lymphocyte differentiation, proliferation, and activity in the immunological system. A new interpretation of elder reports on immunosuppressive effects of aldosterone and aldosterone-antagonists (59) is expected to emerge.

These considerations indicate that extrarenal mineralocorticoid receptors and effector mechanisms may again arouse interest in related fields in clinical science.

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