Aldosterone, like other steroid hormones, initiates its effects by binding to intracellular receptors; these receptors are then able to control the transcription of several genes. The products of these genes eventually modulate the activity of ionic transport systems located in the apical and the basolateral membrane of specialized epithelial cells, thereby modulating the excretion of Na⁺ and K⁺ ions. Considerable progress has been made recently in understanding these mechanisms and the structure of the proteins involved in these processes. A novel principle has been discovered to explain the selective effect of aldosterone on its target epithelia. These tissues exclude competing glucocorticoid hormones by the activity of the 11β-hydroxysteroid dehydrogenase to allow aldosterone, an enzyme-resistant steroid, to bind to its receptors. Aldosterone induces numerous changes in the activity of membrane ion transport systems and enzymes and cell morphology. Although the enhancement of Na,K-ATPase synthesis and the increase of the number of active Na⁺ channels in the apical membrane appear as both direct and primary effects, the mechanism of the other effects remain to be determined. The knowledge of the primary structure of several elements of the aldosterone response system (e.g., mineralocorticoid receptor and Na,K-ATPase) allows us to understand abnormal regulation of Na⁺ balance at the molecular level and, potentially, to identify genetic alterations responsible for these defects. (Hypertension 1992;19:221–227)

KEY WORDS • mineralocorticoid hypertension • sodium transport • aldosterone • carrier proteins • genes

Aldosterone Regulation of Gene Transcription Leading to Control of Ion Transport

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Aldosterone was the first described hormone involved in the regulation of sodium and potassium balance. Isolated deficit of the aldosterone action leads to sodium wasting and hyperkalemia, whereas mineralocorticoid excess induces sodium retention and potassium wasting, although rates of sodium balance and consequently the stability of the extracellular fluid volume and the maintenance of blood pressure. In addition, it also has an important role in regulating the potassium balance. These controls are achieved by adjusting the transport rate of electrolytes across the epithelium lining the distal parts of excretory systems (e.g., distal nephron, colon, and ducts of excretory glands). The scope of the present review will be restricted to the action of aldosterone on these epithelia, the classic target tissues of aldosterone. It should be noticed, however, that high affinity mineralocorticoid receptors have been demonstrated in several nonepithelial cell types. The physiological role of these receptors is not yet completely understood, and for some of these effects, the mechanism of action appears to be completely different from that observed in epithelial cells. However, concerning the regulation of sodium balance, evidence has been provided for a direct involvement of central nervous system receptors in the pathogenesis of mineralocorticoid-induced hypertension.

Mineralocorticoid Effects

The aldosterone-responsive epithelia are able to control the composition of the fluids leaving the organism by establishing large ionic gradients between the internal milieu and the fluid to be excreted. This is possible because they are highly impermeant to passive diffusion of ions (for which reason they are called “tight” epithelia). The effects of aldosterone in mammals have been mostly studied on the cortical collecting tubule of the kidney and on the colon. In these tissues, aldosterone induces both an increase of Na⁺ reabsorption and of K⁺ secretion. The molecular mechanisms of action have been extensively studied in amphibian tight epithelia, for example, the urinary bladder of the toad and more recently in cell cultures originating from these tissues. The amphibian experimental models demonstrate primarily the Na⁺ transport response. The general mechanism by which Na⁺ and K⁺ ions are actively transported across these epithelial cells is represented in Figure 1.

Three phases can be distinguished in the time course of the effect of aldosterone. First, a latent period (30–60 minutes) in which no modification of the Na⁺ transport occurs. Second, an early response (2–3 hours) in which there is a large increase of Na⁺ transport and...
FIGURE 1. Schematic diagram shows mechanism of trans-epithelial Na⁺ and K⁺ transport in "tight" epithelia. The primary active process, the Na-K pump (P), is specifically located in the basolateral membrane (bl m), the membrane facing the blood. It transports Na⁺ ions out of the cell toward the blood and K⁺ ions into the cell. Because of the low intracellular Na⁺ concentration maintained by the Na-K pump, Na⁺ ions enter the cell passively through the Na⁺ channels located in the apical (ap) membrane, facing the luminal fluid. Thus, Na⁺ ions are transported across the cell from the lumen to the blood. K⁺ ions accumulated in the cell by the Na-K pump recirculate across the basolateral membrane through K⁺ channels and, in K⁺-secreting epithelia, also across the apical membrane. In these epithelia, the lumen negative transepithelial potential (−), created by Na⁺ transport, favors the exit of K⁺ into the lumen. The tight junctions (t j) prevent the back-leak of transported ions.

In addition to their effect on Na⁺ and K⁺ transport, mineralocorticoid hormones promote the net secretion of H⁺ ions by the distal nephron and the colon. The mechanism of the increased acid net secretion may result from direct effects such as the stimulation of an N-ethylmaleimide-sensitive H⁺-ATPase or of a H,K-ATPase, or be the consequence of the physiological modifications associated with a higher Na⁺ and K⁺ transport rate such as an increase in luminal negative potential or decrease of the paracellular ionic permeability. The effect of mineralocorticoid on acid secretion can hardly be considered as a regulatory mechanism since blood pH has not been shown to be a stimulus to aldosterone secretion.

The main steps in the mechanisms of action of aldosterone are described in Figure 2 and will be reviewed in the following sections. Similar to other steroid hormones, the first step of aldosterone action is binding to intracellular receptors, and then the hormone-receptor complex interacts with DNA (see "Receptors"). Thereby, it modifies the expression of a number of genes, inducing or repressing the synthesis of several proteins (see "From Receptors to Effectors"). The final effect on electrolyte transport is produced by modification of the function of several membrane transport systems, the effector mechanisms (see "Effector Mechanisms").

Receptors

Binding studies in aldosterone target tissues reveal two distinct binding sites, a type I with a high affinity (Kᵯ around 1 nM) and a low capacity and a type II with a lower affinity (Kᵯ, around 50 nM) and a larger number of binding sites. Because the type I binding site was found specifically in the aldosterone target tissues, it has also been designated as the mineralocorticoid receptor (MR). A MR complementary DNA (cDNA) has been identified and cloned by low-stringency hybridization using a lymphoid glucocorticoid receptor (GR) cDNA as a probe. A new gene encoding a 107 kd protein was found. When expressed in COS cells, the protein encoded by the MR gene had the ability to bind aldosterone and other steroids with affinities similar to those observed for the type I aldosterone receptor. The MR protein is part of a large family of intracellular hormone receptors (e.g., steroid hormone receptor, thyroid hormone receptor, vitamin D receptor, and retinoic acid...
cortisol (R) that share a common general structure, including a central highly conserved (more than 90% homology between members of the family) DNA binding region of about 70 amino acid residues and a C-terminal steroid recognition region.18

Tissue-specific expression of the MR protein is generally restricted to organs in which high affinity aldosterone binding sites have been demonstrated (i.e., the organs including tight epithelia), but MR expression is also observed in the brain (mostly the hippocampus), in the heart, and in blood vessels. The role of the MR in these nonepithelial cells is not yet understood.

The type II aldosterone binding site is generally designated as the GR because its steroid binding characteristics are similar to the GR identified in other tissues.

Both type I and type II demonstrate a poor selectivity between natural glucocorticoid and mineralocorticoid hormones. The MR has a similar affinity (Kₘ around 1 nM) for both aldosterone and cortisol (or corticosterone, the naturally occurring glucocorticoid in the rat),17,19 whereas the GR has only a slightly higher affinity for cortisol (Kₘ, 10–50 nM) than for aldosterone (50–100 nM).19 Considering that total circulating levels of cortisol are usually several orders of magnitude higher than those of aldosterone, and even if cortisol is heavily bound to plasma proteins while aldosterone is mostly free, the free hormone plasma level of cortisol is still many times higher than that of aldosterone. Then how is a mineralocorticoid-specific response to aldosterone possible?

This question has only recently received a satisfactory answer. Aldosterone-responsive epithelia express a high activity of the 11β-hydroxysteroid dehydrogenase (11β-OHSD), an enzymatic complex that catalyzes the transformation of cortisol into the much less active cortisone (Figure 3). Two clinical situations are known in which the activity of this enzyme is reduced. First, the congenital apparent mineralocorticoid excess (AME) syndrome,20 in which 11β-OHSD is deficient. Second, the acquired AME syndrome, which is caused by inhibition with inhibitors of the 11β-OHSD such as glycyrrhetinic acid, found in licorice, or its derivative carbenoxolone, a drug used in the treatment of peptic ulcer.21,22 In both cases, signs of an excessive mineralocorticoid stimulation are observed (e.g., Na⁺ retention, hypertension, and hypokalemia) in spite of low levels of circulating aldosterone and renin. From these observations and the replication of these phenomena in experimental models using carbenoxolone, a new hypothesis has been put forward. In the aldosterone target tissue, the intracellular level of cortisol is maintained at a very low level by rapid metabolism of cortisol into the relatively inactive cortisone by the 11β-OHSD.23 (Figure 3). Corticoid receptors are consequently free to interact with aldosterone, a molecule resistant to the 11β-OHSD activity. Aldosterone is thus allowed to control specifically electrolyte transport through a nonselective receptor, even in the presence of high and variable circulating levels of a potent agonist, cortisol, because this agonist is specifically excluded from the target tissues by enzymatic metabolism. If this mechanism is now well recognized to explain the hypertension of the rare AME hereditary syndrome, the possible role of alteration of the function of the 11β-OHSD in other forms of hypertension still has to be explored.

FIGURE 3. Schematic diagram shows mechanisms of aldosterone selectivity in target tissues. Cortisol is circulating at higher free hormone concentration than aldosterone and has a roughly similar affinity for type I and type II receptors. However, in the "classic" epithelial aldosterone target tissues, cortisol is prevented from binding to mineralocorticoid receptors (for the sake of simplicity only one receptor [R] is drawn in the figure) by rapid metabolism through the 11β-hydroxysteroid dehydrogenase (11β-OHSD). The cortisol metabolite, cortisone, has a much lower affinity for the aldosterone receptors. Conversion of cortisone into cortisol occurs in other tissues, mostly in the liver. Deficient function or inhibition of the 11β-OHSD leads to higher intracellular cortisol concentrations in epithelial target tissues. Cortisol is then able to bind to type I and type II receptors, inducing the signs and symptoms of hyperaldosteronism in the absence of increased aldosterone levels.

The designation of the aldosterone type I as mineralocorticoid receptors and the type II as glucocorticoid receptors in target tissues appears somewhat misleading for several reasons. First, there is the absence of a clear selectivity of the isolated receptor for natural glucocorticoid or mineralocorticoid hormones. Second, occupancy of both types of receptor is necessary for the expression of the full physiological mineralocorticoid response,24 and a mineralocorticoid type response can be elicited in several tissues by specific type II agonists.25,26 Third, if the 11β-OHSD hypothesis is true, type II receptors in aldosterone target tissues would not be exposed to the circulating glucocorticoid hormones unless the capacity of the 11β-OHSD is overcome by high levels of steroids. Until we get a better understanding of the role of each type of receptor, it seems wise to use the "type I" and "type II" aldosterone receptor nomenclature, instead of "mineralocorticoid" and "glucocorticoid" receptor, in aldosterone target tissues.

From Receptors to Effectors

Similar to the other steroid hormones, once aldosterone is bound to its receptor (or receptors), the hormone-receptor complex is able to interact with specific sequences of DNA, the so-called hormone-responsive elements (HRE) to activate or repress specific genes.18,27 Such HRE have been identified for glucocorticoid, progesterone, and estrogen receptors. By analogy, the exis-
tensity of analogous mineralocorticoid-responsive elements has been suspected, and mineralocorticoid receptor–hormone complexes can activate transcription of a reporter gene by binding to the DNA of the glucocorticoid HRE. However, the presence of specific mineralocorticoid response elements in aldosterone-controlled genes of target tissues has still to be demonstrated.

There remains a wide gap in our understanding of the mechanisms that transmit the information from the control of transcription to the effectors; most of them are still hypothetical. The natrierific effect of aldosterone on amphibian tight epithelia is dependent on the synthesis of new proteins, as shown by the inhibition of this effect by protein synthesis inhibition. In mammalian kidney both the natriuretic and the kaliuretic effects can be prevented by protein synthesis inhibitors.

In principle, any of the various membrane transport systems involved in the Na⁺ and K⁺ translocation could be an aldosterone-induced protein (AIP); however, except for the Na⁺-K⁺-ATPase (see below), no other “effector” protein has been characterized as an AIP. Several AIPs have been identified by two-dimensional gel electrophoresis. Among these AIP, a group of glycoproteins (GP70) is expressed during the late phase of the natrierific response and could be localized at or near the apical membrane, suggesting a link to the function of the Na⁺ channel; the function of these GP70 has still to be demonstrated.

Other types of AIP could also act by controlling the activity of the effector proteins by intracellular messengers or by direct protein modifications; indeed, evidence has been provided for the role of protein methylation or phosphorylation in the control of the apical Na⁺ channel. Apical membrane Na⁺ conductance is responsive to cyclic AMP or cyclic GMP, but there is no proof that these mechanisms are involved in the response to aldosterone. Similarly, intracellular Ca²⁺ is known to inhibit the apical Na⁺ channel, but the effect of aldosterone on intracellular Ca²⁺ is not known. Another interesting mechanism, the control of intracellular pH, has been well investigated in amphibian early distal tubule, the physiological equivalent of the mammalian thick ascending limb. In these epithelial cells, aldosterone induces an intracellular alkalinization through the activation of a Na⁺-hydrogen exchange, and since the apical membrane of these segments contains K⁺ channels that can be activated by cytoplasmic alkalinization, this results in an increase of the K⁺ secretion into the tubule lumen. This tubule leak is not a classic tight epithelium aldosterone target; however, aldosterone binding studies as well as immunolocalization studies have revealed mineralocorticoid receptors, although at a comparatively low level. Since physiological studies have been performed with large doses of aldosterone, it is not presently known whether intracellular pH modulation and K⁺ secretion in this segment is mediated by type I or type II receptors, or both. Although intracellular pH also modifies the Na⁺ conductance of tight epithelia, it is not known whether pH mediates the effect of aldosterone in these classic target epithelia.

In addition, aldosterone has been shown to induce an increase of enzyme involved in the generation of ATP, particularly citrate synthase. However, in several amphibian cell lines, a natrierific response could be observed in the absence of detectable change of citrate synthase activity. Whether this metabolic effect of aldosterone is direct or indirect (related to increased Na⁺ transport) is still a matter of debate.

**Effect Mechanisms**

As outlined in Figure 1, at least four basic elements are involved in the vectorial active transport of Na⁺ across a tight epithelium. First, the apical Na⁺ channel provides a regulated permeation pathway of Na⁺ across the apical membrane. Second, the basolateral Na⁺-K⁺-ATPase, or Na⁺-K⁺ pump, constitutes the Na⁺ pathway across the basolateral membrane and, using the metabolic energy supplied in the form of ATP, maintains the low intracellular Na⁺ concentration that drives this ion from the lumen into the cell. Third, because the Na⁺ ions are pumped by the Na⁺-K⁺-ATPase in exchange for K⁺ ions, a pathway must exist to allow K⁺ to exit the cell. K⁺ channels are present in the basolateral membrane of all tight epithelia. In some tight epithelia (for which the main function is Na⁺ reabsorption), these basolateral K⁺ channels constitute the main pathway of K⁺ recirculation. In other tight epithelia, those involved in the secretion of potassium (the cortical collecting tubule and, to a lesser extent, the distal colon), K⁺ channels are also present in the apical membrane, allowing K⁺ to exit the cell toward the apical compartment. Finally, the tight junctions, the structure controlling the paracellular pathway, must be impermeant to Na⁺ ions to prevent the back leak of Na⁺ from the basolateral to the apical compartment. The effects of aldosterone on each of these elements will be reviewed in the following paragraphs.

Entry of Na⁺ across the apical membrane through Na⁺ channels is the rate-limiting step of transepithelial Na⁺ transport in tight epithelia. The control of the number of active Na⁺ channels in the apical membrane thus appears as the first possible target for a mineralocorticoid hormone. The epithelial amiloride-sensitive Na⁺ channel is a complex protein structure: the purified amiloride binding membrane protein isolated from bovine kidney medulla is composed of six distinct subunits ranging in molecular weight from 40 to 315 kDa. Although amiloride seems to bind to a 150-kd polypeptide, the function of each subunit has yet to be determined. The physiological characteristics of single amiloride-sensitive Na⁺ channels have been studied by the patch clamp technique, and they appear similar in mammalian and amphibian tight epithelia: the channel has a small conductance of about 5 picoamperes and slow kinetics (mean open time in the second range). In contrast with the Na⁺ channel of excitable cells, the epithelial Na⁺ channel is poorly voltage-sensitive, with a slight activation by membrane hyperpolarization. A number of studies have identified the increase of the apical membrane Na⁺ permeability as the main event of the early response to aldosterone (see Reference 9 for review). This effect is due to an increase of the number of active channels without change of the conductance of each channel. Recent data indicate that the kinetics of opening and closing of the channel could be modulated by aldosterone. Although the Na⁺ channel may seem a likely candidate for an AIP, it appears that at least part of the proteins constitutive of the channel are already present in the apical membrane.
before aldosterone action. Previous exposure of the apical membrane to pro tease enzymes or carbox yle-reactive agents prevents the subsequent effect of aldoster one(60-63) (in contrast with the effect of ADH, which is thought to act by addition of new membrane-containing Na+ channels and is not prevented by the same maneuver), and the pool of Na+ entry is not altered by aldosterone. This is confirmed by the absence of an effect on aldosterone on the amount of message RNA (mRNA) coding for amiloride-sensitive Na+ channels in Na+ transporting A6 cells. Asher and Garty have been able to demonstrate two phases in the effect of aldosterone on the apical membrane Na+ conductance in the toad bladder. During the early phase (3 hours), although aldosterone induced a more than twofold increase of the apical membrane permeability to Na+, vesicles isolated from the epithelium do not demonstrate an increase of Na+ transport; thus, this early effect is lost in the process of isolating the membrane. In contrast, after a longer exposure to aldosterone (6 hours), resulting in an additional increase of apical membrane permeability, an increase of amiloride-sensitive Na+ transport was observed in the vesicles; this later change apparently results from a permanent alteration of the Na+ channel.

The Na-K pump has the best characterized structure of these four elements. Its primary structure has been determined in humans and in several species. The functional Na,K-ATPase is composed of two subunits. The first is a 100-kd a-subunit that contains the catalytic sites of ATP hydrolysis, the Na+ and K+ transport binding sites, and the binding site for the specific inhibitors the cardiac glycosides. The second is a 40-60-kd glycosylated b-subunit, the function of which is not entirely determined, but it is at least involved in the maturation and expression to the surface of the a-subunit. Of the three known isoforms (a1, a2, a3), only the a1 isoform is expressed in significant amounts in epithelial cells. Two b isoforms are present in amphibian tight epithelial cells, but only the b1 isoform expression is induced by aldosterone. The Na,K-ATPase has been definitely identified as one of the A1Ps in amphibian tight epithelia. Aldosterone induces a rapid (detectable within 15 minutes) increase of the transcription of both the a- and the b-subunit genes. This leads to a twofold to fourfold accumulation of the mRNA coding for both subunits. The rapidity of this effect favors the hypothesis that both genes are directly regulated by a corticosteroid-responsive element, although a more complex mechanism cannot be excluded. At the protein level, it was shown that the synthesis of both a- and b-subunits was increased after exposure to low doses of aldosterone corresponding to occupancy of type I receptors, although maximal effect can be obtained only by using large concentrations of aldosterone corresponding to occupancy of type II receptors. This effect is not apparent before the third hour of aldosterone exposure and thus appear to be an event of the late response. Because this response occurs even after inhibition by amiloride of Na+ entry through the apical membrane, it is not only a consequence of an increase Na+ load to the cell. On the other hand, several investigators have observed that preventing Na+ entry by blocking apical membrane Na+ channels reduced the effect of aldosterone on the activity of the basolateral Na-K pump, whereas others found opposite evidence. These apparently contradictory results may be reconciled by the recent observation by Blot-Chabaud et al and Barlet-Bas et al that aldosterone controls a latent pool of Na-K pump and that active Na-K pump are recruited from this pool when the sodium load to the cell is increased. Thus, although aldosterone induces directly the synthesis of new Na,K-ATPase units, an increase of active Na-K pump at the plasma membrane might be observed only if an adequate Na+ loading of the cell is allowed.

Aldosterone effects may include an activation of both apical and basolateral K+ channels. An increase of the basolateral K+ conductance has been reported both in mammalian and in amphibian tight epithelia. Although the type of K+ channels involved in this response has not been determined, they appear to be lidocaine-sensitive. In toad bladder cells, the increase of the basolateral K+ conductance occurred during the early response and was independent of the increase of Na+ transport.

In the K+-secreting epithelia such as the cortical collecting tubule, the stimulation of K+ secretion by aldosterone can be explained by several mechanisms. First, the apical membrane is depolarized by the increased Na+ permeability, leading to an increase of the driving force for K+ exit from the cell to the lumen. Second, the increase of the basolateral Na-K pump activity hyperpolarizes the basolateral membrane potential preventing K+ exit to the basolateral side and accumulates more K+ into the cell. Third, the increase of the basolateral Na-K pump at the plasma membrane might be amplified by the secretory K+ flux due to the increased driving force. Such an increase of the apical K+ conductance has been well demonstrated in the cortical collecting tubule of the rabbit. In this model, this effect was part of the late response and appeared to be dependent on Na+ transport. The relative importance of the change of driving force versus change of conductance for K+ across the apical membrane is still a matter of debate and may be species specific.

The tight junctions may also be a target of the action of aldosterone. Several investigators have reported an increase of the paracellular resistance of the rabbit cortical collecting tubule or the colon after exposure to mineralocorticoid. In contrast, aldosterone increased the paracellular permeability in A6 cells, an effect that was dependent on Na+ transport. These effects were relatively small, and their physiological significance is not known.

**Perspectives and Conclusions**

Blood pressure is a quantitative trait that varies continuously in the whole population. Blood pressure and its regulation are controlled by a variety of mechanisms involving probably several genetic loci and environmental and other factors, such as diet, body weight, stress, and physical exercise. The hereditary nature of hypertension has been demonstrated in both human and animal models. As pointed out by Corvol and colleagues, two main approaches have been used to
study the genetic factors involved in arterial hypertension. One approach is to test a series of genetic markers distributed throughout the genome to carry a link between increased blood pressure and specific genes. The other approach is to study candidate genes that are known to participate in blood pressure regulation, such as hormonal factors (e.g., renin-angiotensin-aldosterone axis, catecholamines, and vasopressin), factors involved in target cells (e.g., heart and blood vessels), or as described in this short review, the distal segments of the nephron, which play a critical role in the fine control of sodium metabolism. It is quite evident that the control of sodium reabsorption in the distal nephron could be one of the first factors in the establishment of hypertension. The link between sodium metabolism and hypertension has been often discussed, and in most physiological models that have been proposed, a "kidney factor" has been postulated to explain the genesis of high blood pressure.

We believe that the identification of the sodium transport protein involved in sodium reabsorption in the distal nephron and the identification of the molecular mechanisms by which these transporters are controlled should lead to the identification of candidate genes playing a role in the genesis of hypertension. Among other congenital diseases, the congenital deficiency in 11β-OHSD is a recent example of a possible candidate gene involved in essential arterial hypertension. In the future, it will be of special interest to understand in molecular terms the entry of sodium at the apical membrane through amiloride-sensitive sodium channels, which is the limiting factor in the sodium transport function of tight epithelia. In 1963, Liddle and his colleagues described a familial hypertension disorder simulating primary aldosteronism but with negligible aldosterone secretion. This disorder could be corrected by treatment with low doses of a sodium channel inhibitor, suggesting that the sodium channel itself (or its regulation) was involved. In this context, we believe that the understanding of the molecular mechanism by which aldosterone controls sodium reabsorption is a prerequisite in defining the most important candidate genes involved in the genesis of hypertension linked to renal sodium retention.

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Horisberger and Rossier  
Aldosterone Regulation of Ion Transport  
227


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J D Horisberger and B C Rossier

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