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MECHANISM OF TRANSPORT ACTIVATION

Physiologic resistance to the action of aldosterone

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Physiologic resistance to the action of aldosterone. The collecting duct is one of the major targets for aldosterone's action. Experiments conducted several years ago suggested that the major site of action on Na^+ and K^+ transport was the cortical portion, the cortical collecting duct (CCD). Subsequent studies have shown that the entire collecting duct is capable of responding to aldosterone, but does so differently according to the region. The inner medullary collecting duct (IMCD), while exhibiting a relatively low rate of Na^+ transport in isolated, perfused tubules, can develop substantial rates of Na^+ transport when put in primary culture. The IMCD, in contrast to the CCD, usually secretes little K^+. Investigations into the mechanisms for the lower rates of Na^+ transport have revealed that transforming growth factor-β (TGF-β), which is endogenously produced in the inner medulla, can markedly reduce the natriferric action of aldosterone. This action of TGF-β is not apparent within the first few hours of exposure, but its effects, even after removal, last for over 48 hours. The mechanism of this antagonism appears to involve pathways that are parallel and independent of the major transcriptional effects of aldosterone.

Ever since the discovery of aldosterone and the recognition that it served to increase greatly the rate of Na^+ absorption across high-resistance epithelia, enormous efforts have been expended in delineating the molecular mechanisms of its action. The application of modern molecular techniques have greatly accelerated the pace of our understanding of aldosterone's action. Analyses using electrical, biophysical, and molecular measurements have now confirmed that one of its major effects on epithelia is to increase the entry of Na^+ across the apical membrane via the epithelial Na^+ channel (ENaC). Despite these fundamental advances, the precise mechanisms by which aldosterone produces its effect remain incompletely understood. To get a better understanding of the molecular mechanisms and to provide an expanded framework from which to view aldosterone's actions, we have begun to focus on circumstances in which aldosterone does not exert its full effect. The major purpose of this line of investigation is to understand better how aldosterone works and how its actions can be counter-regulated.

A large number of vasoactive agents have been found to be effective in reducing Na^+ transport by the cortical collecting duct (CCD), toad urinary bladder, and model epithelial monolayers [1–3]. These models have produced considerable insight into intracellular mechanisms responsible for acute regulation of ENaC. Less attention has been focused on longer term regulation of Na^+ transport. The reason for this relative lack of attention is primarily one of convenience. Before the cell culture systems became widely used and reliable, measurements of Na^+ transport were more readily accomplished over a short period of time (up to 30 min) compared with longer times (hours to days). In this review, the evidence supporting the idea that physiologically important regulatory systems exist that can, in effect, render the collecting duct (or target epithelia) unresponsive to the actions of aldosterone is suggested.

HETEROGENEITY OF THE COLLECTING DUCT

Both superficial and deep nephrons empty into the CCD near the surface of the kidney, whereupon it descends through the cortex, outer medulla, and finally inner medulla to empty into the ducts of bilini and the renal pelvis. At one time, it was believed that the major function of the collecting duct was similar along its length, but we have learned in the past several years that this notion is overly simplistic. While the capability of absorbing water in response to vasopressin is a feature shared by all segments of the collecting duct, the capabilities for solute transport are quite different. One of the earliest examples in which the capabilities of ion transport along the collecting duct were recognized as being quite different is shown in Figure 1. In these experiments, rabbits were treated with a mineralocorticoid hormone for several days, and the collecting duct was dissected from various regions within the cortex and outer medulla. The position of the collecting duct and its orientation were carefully recorded and plotted as a function

Key words: aldosterone, transforming growth factor β, collecting duct, ENaC.

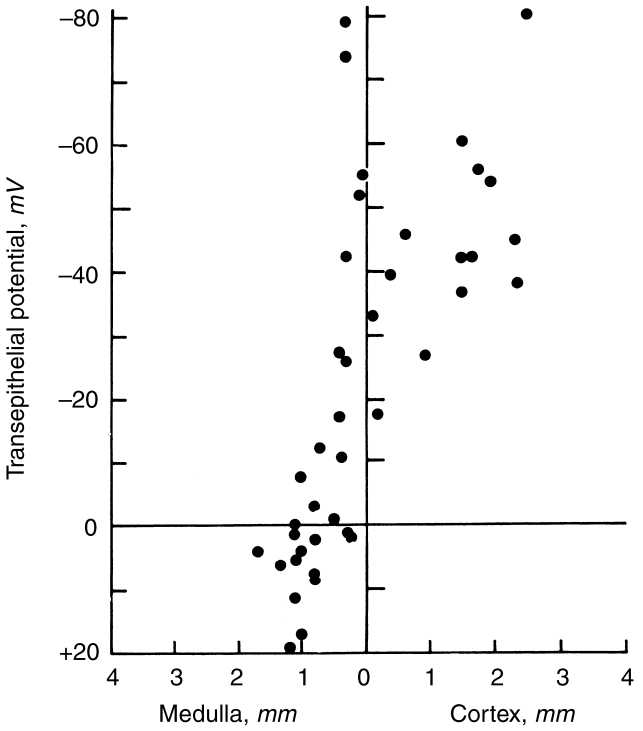

Fig. 1. Transepithelial voltage across the cortical collecting duct of the rabbit. Rabbits were treated with mineralocorticoid hormone for several days, and collecting ducts were dissected from specific regions of the kidney and perfused in vitro. The greater the absolute value of the lumen-negative voltage, the greater the magnitude of Na^+ transport across the collecting duct became [23]. The cortical collecting duct transports substantially more Na^+ than does the medullary collecting duct, even under maximal mineralocorticoid stimulation (reproduced with modifications from Stokes, Tisher, and Kokko, with permission from the International Society of Nephrology) [24].

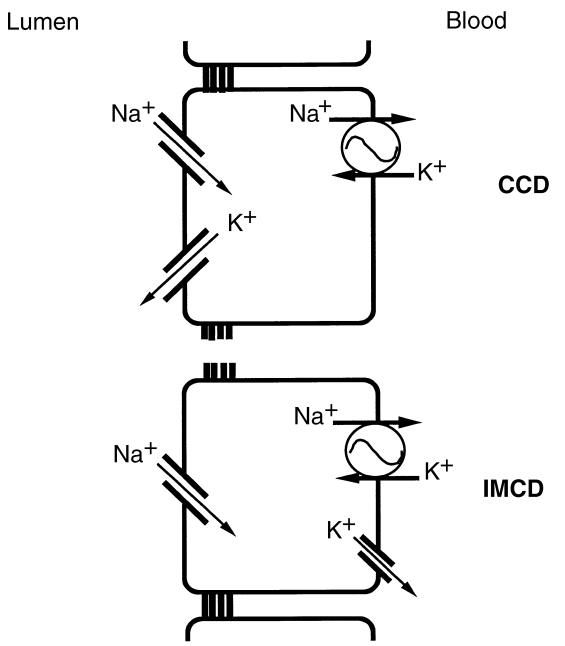

Fig. 2. Schematic of cortical collecting duct (CCD) and inner medullary collecting duct (IMCD) Na^+ and K^+ transport pathways.

of the transepithelial voltage measured by perfusion of that segment. As shown on the right-hand side of the panel, virtually all of the tubules dissected from cortex (that is, the CCD) display spontaneously lumen-negative voltage. Subsequently, it has been well described that this lumen-negative voltage is produced by active Na^+ transport through the ENaC located on the apical membrane. This transport system is coupled in series to the Na,K-ATPase located on the basolateral membrane. Figure 1 also shows that collecting ducts from deeper in the kidney and into the outer medullary collecting duct are different; the magnitude of the voltage falls dramatically. Within the inner stripe of the outer medulla, the transepithelial voltage is almost always lumen positive. Changes in two transport systems are responsible for this change in voltage. First, there is an increase in the magnitude of electrogenic proton secretion. Second, there is a large reduction in the magnitude of Na^+ transport. The discovery that despite intensive mineralocorticoid therapy Na^+ transport is much lower in the medullary collecting ducts than in CCDs led some people to speculate that this region of the nephron was not sensitive to aldosterone. This notion turned out to not be completely correct.

The collecting duct, all along its length, contains the machinery to respond to aldosterone and to absorb Na^+. Three molecular complexes are necessary for this aldosterone response: mineralocorticoid receptors, ENaCs, and Na/K pumps. Each of these is present along the length of the collecting duct. Earlier work from micropuncture studies has clearly demonstrated that the inner medullary collecting duct (IMCD) is capable of absorbing Na^+ actively [4]. Furthermore, when IMCD cells are isolated from rat and cultured on permeable supports, these cells transport Na^+ much like the CCD and the toad urinary bladder. The major differences between the Na^+ absorption process in the CCD and the IMCD are shown in Figure 2. The CCD secretes K^+ because of the large number of K^+ channels on the apical membrane. In contrast, the IMCD secretes little K^+ because there are few, if any, K^+ channels on the apical membrane. The major active transport process affected by the IMCD is NaCl absorption.

This difference between the CCD and the IMCD has physiologic importance. Aldosterone's actions on the CCD produce both Na^+ absorption and K^+ secretion. However, its actions on the IMCD would not affect K^+ secretion to a significant degree and would be almost exclusively focused on NaCl absorption. Thus, the extent of the actions of aldosterone, widely known to promote both NaCl retention and K^+ secretion, is a function of where they are exerted.

Despite the fact that all of the machinery is present in the IMCD to respond to aldosterone by increasing Na^+ transport, efforts to study the regulation of Na^+ transport by aldosterone in this segment have been difficult. When IMCD segments from rabbit and rat are dissected from animals having received mineralocorticoid hormones and are perfused in vitro, the magnitude of Na^+ transport is surprisingly low [5–8]. To study the mechanisms of Na^+ transport regulation by steroids and other hormones and cytokines, we developed the technique of primary cultures of the IMCD from rat. When grown on polycarbonate filters, these monolayers look very similar to the native IMCD cells and have similar transport characteristics [7].

EVIDENCE FOR ALDOSTERONE RESISTANCE

In the process of attempting to describe Na^+ transport by primary IMCD cultures, we discovered that if serum were added to a serum-free culture medium at the time that aldosterone was added, the increase in Na^+ transport did not occur [9]. These results were surprising and, at the time we discovered them, unprecedented. The results were even more surprising when we discovered that if serum was removed after exposing the cells for 24 hours but the exposure to aldosterone continued, the monolayers did not increase their Na^+ transport. These results indicated that a factor(s) in serum was able to confer resistance to the action of aldosterone. Furthermore, this action was not confined to the period of exposure but was relatively long lasting.

In pursuing this important lead, we evaluated a large number of hormones and cytokines for which there was evidence of action on collecting ducts and presence in the inner medulla. To our surprise, a number of peptide hormones that might have been predicted to produce an effect did not do so. Such hormones as vasopressin, atrial natriuretic peptide, endothelin, epidermal growth factor, insulin-like growth factor 1, and a large number of other agents had no effect. To date, only two have been discovered that produce results similar to that of serum. These agents are transforming growth factor-β (TGF-β) and interleukin-1. We have studied the effects and interactions of aldosterone and TGF-β in greater detail than those with interleukin-1.

INTERACTIONS OF STEROID HORMONES AND TRANSFORMING GROWTH FACTOR-β ON INNER MEDULLARY COLLECTING DUCT CELLS

Inner medullary collecting duct (IMCD) monolayers treated with aldosterone show an increase in the short circuit current (a precise measure of Na^+ transport) by threefold to fourfold. In contrast, monolayers treated with aldosterone and TGF-β for 24 hours show minimal increase in the Na^+ transport rate [9]. However, TGF-β has no acute (up to 2 h) effect on Na^+ transport. The earliest one can demonstrate a modest reduction in Na^+ transport is three hours, with the full effect of TGF-β seen after 12 to 24 hours. This time course suggests a mechanism that involves gene transcription and protein synthesis. It is quite different from agents that act within minutes via signaling cascades that involve protein kinase activities [1, 3, 10, 11].

Both mineralocorticoid hormone and glucocorticoid hormone, acting through their cognate receptors, can produce equal increases in Na^+ transport by IMCD cells [12]. TGF-β inhibits the action of either hormone; however, its actions are specific for Na^+ transport because TGF-β does not modify the ability of IMCD cells to increase Cl secretion in response to cAMP agonists [13]. In addition, TGF-β has no effect on protein, DNA, or adenosine 5'-triphosphate (ATP) content [9].

The time course of TGF-β's action is also quite interesting. If monolayers are exposed to TGF-β for only three hours before they are treated with aldosterone, the cells fail to increase their Na^+ transport for over 48 hours (for as long as electrical properties remain intact). Thus, TGF-β seems to act like a switch. It appears to activate (or inactivate) a series of cell processes that render the IMCD cell incapable of increasing Na^+ transport in response to steroid hormones.

PHYSIOLOGICAL RELEVANCE OF TRANSFORMING GROWTH FACTOR-β AND ALDOSTERONE INTERACTION

It is well established that conditions of inflammation will often stimulate the production of TGF-β. It is less well appreciated that TGF-β is present in certain normal organs and tissues in relatively high abundance. In the kidney, the amount of TGF-β present in the normal cortex is quite low, in contrast to circumstances of glomerulonephritis. However, the amount of TGF-β mRNA present in the inner medulla is quite high [14], and the amount of protein demonstrable by immunocytochemistry is higher in the inner medulla than in the cortex [15]. The relatively large amount of TGF-β1 present in the inner medulla may be one reason that Na^+ transport by the normal IMCD is substantially lower than that of the CCD.

Transforming growth factor-β may serve as a dynamic regulator of the action of aldosterone with respect to Na^+ transport. Ying and Sanders demonstrated that feeding rats a high-salt diet caused an increase in TGF-β1 synthesis and excretion [16]. Thus, an increase in TGF-β production induced by a high-salt diet might increase the resistance to aldosterone by the IMCD. Viewed from this perspective, TGF-β could be one of the factors responsible for the escape phenomenon from chronic min-

eralocorticoid hormone stimulation. This interaction between TGF-β and aldosterone may also be important in certain pathological states leading to salt wasting and hyperkalemia. High circulating aldosterone levels with salt wasting have been described in some models of interstitial nephritis and obstructive uropathy [17]. In such cases, TGF-β might be overproduced even in the cortex and thus produce resistance to aldosterone all along the collecting duct.

MECHANISM OF ACTION OF ALDOSTERONE AND TRANSFORMING GROWTH FACTOR-β ON THE INNER MEDULLARY COLLECTING DUCT CELLS

Aldosterone increases electrogenic Na^+ transport by increasing the activity of ENaC. It is becoming increasingly clear that the molecular effects of aldosterone are cell specific. One dramatic example is the difference between the stimulation of Na^+ transport in the colon compared with the collecting duct. In the colon, aldosterone stimulates the steady-state mRNA levels of the β and γ subunits of ENaC, while in the collecting duct, aldosterone stimulates steady-state levels of the α subunit and not the β and γ subunit. The molecular basis for this differential response to aldosterone is not clear at the present time. Also unclear are the mechanisms responsible for intrarenal heterogeneity of the responses. Pácha et al have shown that feeding rats a low-Na^+ diet increases the activity of ENaC in CCD segments [18]. However, recent work from our laboratory has shown that this low-Na^+ diet, which increases the amount of β and γ ENaC mRNA in colon, does not change the steady-state mRNA levels of any of the ENaC subunits in the renal cortex [19]. This lack of response of steady-state mRNA levels in cortex contrasts dramatically with the response in the inner medulla. A low-salt diet increases α ENaC mRNA in this region but has no effect on β or γ ENaC mRNA. These regional differences in response to dietary salt intake underscore important functional differences between the CCD and the IMCD.

The action of aldosterone on the function of the Na/K pump has been the subject of considerable investigation with some inconsistent results. In the IMCD in primary culture, we have found that whereas the Na^+ transport rate increases by threefold to fourfold, the magnitude of the increase in Na,K-ATPase is minimal [20]. Aldosterone increases the maximal capacity of the Na/K pump, as measured under conditions of permeabilization of the apical membrane, by about 30%. We have also demonstrated that aldosterone increases the ouabain binding in IMCD cells. These results implicate a process whereby aldosterone activates Na/K pumps. One possibility is that pumps are translocated from an inactive intracellular site to the basolateral membrane [20]. The effect of aldosterone on Na^+ transport appears to be greater on ENaC than on the Na/K pump. By taking the ratio of Na^+ transport and the maximum capacity of the pump, one can show that under normal circumstances (without aldosterone), the pump operates at about 20% of its maximum capacity. However, following aldosterone stimulation, the pump operates at about 50% of its maximum capacity. The explanation for this difference is probably that the increase in the entry of Na^+ across the apical membrane increases the intracellular Na^+ concentration, which by its kinetic effect increases the rate of extrusion of Na^+ via the Na/K pump. We have calculated that the effect of aldosterone on intracellular Na^+ given the measured kinetics of the pump in IMCD cells would increase the Na^+ concentration by about 2 mmol/L [21].

Table 1. Effects of aldosterone and transforming growth factor-β (TGF-β) on Na^+ transport by primary cultures of rat inner medullary collecting duct

	I_{Na}	I_{pump}	α_1-Na,K-ATPase	α-ENaC
Aldosterone	↑↑↑	↑	↑	↑↑↑
Aldosterone + benzamil	↓	↑	↑	↑↑↑
Aldosterone + TGF-β	↔	↔	↔	↑↑↑

Abbreviations are: I_{Na}, sodium current across the monolayer; I_{pump}, maximum current generated by the Na,K-ATPase in intact monolayers; α_1-Na,K-ATPase; mRNA abundance of the α_1 subunit of the Na/K pump; α-ENaC, mRNA abundance of the α subunit of the epithelial Na^+ channel. Arrows indicate changes from monolayers not treated with aldosterone.

FUNCTIONAL AND MOLECULAR INTERACTIONS OF ALDOSTERONE AND TRANSFORMING GROWTH FACTOR-β

To understand the mechanisms whereby TGF-β antagonizes the actions of aldosterone, it is necessary to first understand how aldosterone affects IMCD cells when an increase in Na^+ transport is prevented. To do so, we tested the effects of aldosterone on cells treated with a low concentration of benzamil, an amiloride analogue. Benzamil did not prevent the increase in the capacity of the Na/K pump. Benzamil likewise did not prevent the modest increase in the mRNA levels of the α subunit of the Na/K pump (Table 1). Thus, these effects of aldosterone on the pump appear to be direct and not secondary to the increase in Na^+ transport effected by aldosterone.

The actions of TGF-β on these parameters are also shown in Table 1. TGF-β prevented aldosterone's stimulation of the capacity of the Na/K pump. Furthermore, it prevented the modest increase in the mRNA abundance of the α subunit of the Na/K pump. However, TGF-β had no effect on the stimulation of the αENaC mRNA abundance.

These results demonstrate complex interactions between TGF-β and aldosterone. Whereas TGF-β can in-

terrupt the actions of aldosterone to stimulate the Na^+ pump activity, they do not do so by interfering with the receptor machinery for aldosterone's action. The failure to inhibit aldosterone's stimulation of αENaC mRNA clearly demonstrates that TGF-β does not interfere with aldosterone's interaction with the mineralocorticoid receptor, to its translocation to the nucleus, or to the increase in the transcription of the αENaC mRNA [22]. The mechanisms whereby TGF-β reduces the actions of aldosterone are probably multifactorial and likely involve parallel actions in the regulation of the activity of ENaC.

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