Regulation of the Na-K Pump of the Rat Cortical Collecting Tubule by Aldosterone

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ABSTRACT Activities of Na channels and Na pumps were studied in the rat cortical collecting tubule (CCT) during manipulation of the animals' mineralocorticoid status in vivo using a low-Na diet, diuretics, or administration of exogenous aldosterone. Tubules were isolated and split open to expose the luminal membrane surface. Using the whole-cell patch-clamp technique, activities of the apical Na channels and the basolateral Na pumps were measured in principal cells as the currents inhibited by amiloride (10 μM) and ouabain (1 mM), respectively. Na channel current (INa) was not measurable in CCTs from control animals on a normal diet. INa was ~200 pA/cell in CCTs from animals on a low-Na diet or infused with aldosterone using osmotic minipumps. Currents attributable to the Na pump (Ipump) were similar in control animals and animals on a low-Na diet. Maximal currents were ~35 pA/cell in both groups, and decreased with hyperpolarization of the cell membrane. In contrast, administration of exogenous aldosterone increased Ipump fourfold. Coinfusion of aldosterone and amiloride in vivo through the minipumps did not affect the induction of INa but reduced the induction of Ipump by 80%. We conclude that the induction of channel activity in this tissue is a direct action of aldosterone, whereas the induction of pump activity may be a consequence of the increased Na traffic through the epithelial cells.

INTRODUCTION

As discussed in the companion paper (Pácha, Frindt, Antonian, Silver, and Palmer, 1993), epithelial Na channels are clearly under the control of the mineralocorticoid aldosterone in a number of tissues, including the rat renal cortical collecting tubule (CCT). The possible stimulation of the basolateral Na pump in response to aldosterone has been more controversial. In amphibian epithelia, there is strong evidence for the induction of the Na pump by aldosterone during the so-called late phase of the hormone's action (3–24 h after administration) (Verrey, Schaerer, Zoerkler, Paccolat, Geering, Kraehenbuhl, and Rossier, 1987; Verrey, Kraehenbuhl, and Rossier, 1989). In the mammalian kidney, chronic elevation of aldosterone in vivo increases the Na,K-ATPase activity, particularly in the CCT. There is controversy, however, on the extent to which this induction is due to direct effects of the steroid or
to increases in cell Na subsequent to increased Na permeability (Katz, 1990; Marver, 1992). More recently, short-term effects of aldosterone on Na,K-ATPase activity have been reported, some of which require increases in Na entry to be expressed (Barlet-Bas, Khadouri, Marsy, and Doucet, 1990; Blot-Chabaud, Wanstok, Bonvalet, and Farman, 1990).

The purpose of the study reported in this article was to compare the responses of Na channels and Na pumps to changes in mineralocorticoid status. We have used the whole-cell variation of the patch-clamp technique to measure the activities of Na channels and Na pumps in the principal cells of rat CCT in vitro after subjecting the animals to a variety of experimental conditions in vivo.

**MATERIALS AND METHODS**

**Biological Preparations**

Sprague-Dawley rats of either sex (100–150 g) raised free of viral infections (Charles River Laboratories, Wilmington, MA) were fed either a normal rat chow or a low-Na diet (ICN, Biochemicals, Cleveland, OH) as described in the previous paper. To increase the levels of circulating aldosterone directly, the animals were implanted subcutaneously with osmotic minipumps (model 2002; Alza Corp., Palo Alto, CA). The pumps were filled with aldosterone (Sigma Chemical Co., St. Louis, MO) dissolved in polyethylene glycol 300 at concentrations designed to provide rates of infusion of 100–500 (µg/kg body wt)/d. These rates were chosen to achieve plasma aldosterone levels of 100–500 ng/dl, based on previously published data (Will, Cortright, DeLisle, Douglas, and Hopfer, 1985). Measured values agreed well with these estimates (Table I).

In some experiments amiloride (Research Biochemicals Inc., Natick, MA) was added to the minipumps at a concentration of 60 mM to give an infusion rate of 500 pmol/min. This rate was chosen to give a steady-state concentration of the drug of ~10 µM in the tubular fluid of the CCT. We assumed that the glomerular filtration rate was 1 ml/min, that 5% of the filtered fluid volume reached the CCT, and that all of the amiloride is unmetabolized and excreted by the kidneys (Baer, Jones, Spitzer, and Russo, 1967). These animals were also given a bolus injection of amiloride of 5 mg i.p. to saturate amiloride-binding stores.

The CCTs were prepared for patch-clamp studies as described in the companion paper (Pacha et al., 1993). Measurements of whole-cell currents were carried out at 37°C by superfusing the tubules with fluid prewarmed with a miniature water-jacketed glass coil.

**Table I**

| Condition                        | Plasma aldosterone | No. of observations |
|----------------------------------|--------------------|---------------------|
| Control                          | 16 ± 4             | (7)                 |
| Low-Na diet (7–14 d)             | 1,260 ± 130        | (8)                 |
| Aldosterone infusion, 100 µg/kg · d | 110 ± 25           | (4)                 |
| Aldosterone infusion, 500 µg/kg · d | 540 ± 190          | (3)                 |
| Aldosterone + amiloride infusion, 200 µg/kg · d + 220 µg/d | 300 ± 120          | (3)                 |
| Amiloride infusion, 220 µg/d     | 82 ± 36            | (3)                 |
Solutions

Tubules were superfused with a solution consisting of (mM): 140 Na methanesulfonate, 5 K methanesulfonate, 2 CaCl₂, 1 MgCl₂, 2 glucose, and 10 HEPES, adjusted to pH 7.4 with NaOH. In some experiments 5 mM Ba acetate was added to reduce the membrane K conductance. Low Cl concentrations were used to reduce Cl conductance. Amiloride was dissolved in deionized water at a concentration of 10 mM and was added to the superfusate at a final concentration of 10 μM. Ouabain was dissolved directly in the superfusate at a concentration of 1 mM. The patch-clamp pipettes were filled with solutions containing (mM): 80 Cs, 50 Na, 20 tetraethylammonium, 3 Mg, 120 aspartate, 6 Cl, 10 EGTA, 10 HEPES, and 10 MgATP. This solution was adjusted to pH 7.5 with NaOH. This solution is similar to that used previously (Gadsby, Kimura, and Noma, 1985) to study Na pump currents in the heart. Cs and aspartate were used as the major cation and anion to reduce K and Cl conductances, facilitating the measurement of pump currents. We assume that 50 mM intracellular Na is sufficient to fully activate the pump, and that Cs does not affect the pump currents.

Electrical

Basic whole-cell patch-clamp methods were as described previously (Frindt et al., 1990; Pácha et al., 1991). After formation of the seal the membrane patch was ruptured with increased suction, resulting in a sudden change in current due to the cell potential and increase in the size of the capacitative transients resulting from 20-mV voltage steps. It was previously shown (Frindt et al., 1990), using a fluorescent dye, that the cells of the CCT are not coupled by gap junctions, so that individual cells within an epithelium can be studied using this method. Recording of currents and analysis of data were carried out on an Atari 1040 ST computer equipped with interface and data acquisition software (Instrutech, Mineola, NY). Current records were stored on video tape using a pulse-code modulator (Instrutech). Estimates of membrane capacitance were made from the current transients in response to a 20-mV voltage step as described previously (Frindt et al., 1990). The equation used to calculate the capacitance was:

\[ I(t) = \frac{(\Delta V)}{R_m} \left[ 1 - \frac{1}{1 + \beta} \right] e^{-\beta t} \]

where \( \Delta V \) is the size of the voltage step, \( R_m \) and \( R_s \) are the resistances of the membrane and of the pipette (series resistance), respectively, \( \beta = \frac{R_m}{R_m + R_s} \), and \( C_m \) is the membrane capacitance. Note that there is a typographical error in this equation in our previous description of this method (Frindt et al., 1990).

Plasma Aldosterone

Plasma aldosterone concentrations were measured using radioimmunoassay as described in the companion paper (Pácha et al., 1993).

RESULTS

Fig. 1 shows typical current tracings under whole-cell clamp conditions as the pipette potential is changed between +40 and −60 mV. The two traces shown are from the same cell before and after addition of 10 μM amiloride to the superfusate. There was
no significant time dependence of the currents in response to voltage changes, other than the capacitative transients, in either the absence or the presence of amiloride. The effect of amiloride was usually complete within 30–40 s after initiation of the solution change, and was reversible (not shown). The steady-state $I-V$ relationships for both conditions are shown in Fig. 1 B. Subtraction of the currents with and without the blocker gives the amiloride-sensitive current ($I_{Na}$) as a function of voltage, shown as filled symbols in Fig. 1 B. $I_{Na}$ is negative, representing inward current, at
Figure 2. Whole-cell currents in the presence and absence of ouabain. (A) Records are from the same cell shown in Fig. 1. Amiloride (10⁻⁵ M) and Ba acetate (5 mM) were present in the bath throughout to block Na and K conductances, respectively. Pulse protocol was identical to that in Fig. 1. (B) I-V relationships are shown just before ouabain (+Ba) (triangles), ~1 min after addition of 1 mM ouabain (circles), and 3 min after removal of ouabain (inverted triangles) from the perfusate. The ouabain-sensitive currents (Ipump) are shown as filled squares.
zero and negative cell potentials, and reverses at cell potentials between +20 and +30 mV. This is consistent with the calculated Nernst potential for Na across the cell membrane of 25 mV. These results are similar to those described in an earlier study (Frindt et al., 1990) except for the less positive reversal potential, which is accounted for by the relatively high Na concentration in the pipette, which presumably equilibrates with the cytoplasm under the whole-cell clamp conditions. Because the cells of the rat CCT are not coupled by gap junctions (Frindt et al., 1990) these currents indicate the Na channel conductance of a single principal cell.

The activity of the Na/K pump in the rat CCT was estimated as the whole-cell current sensitive to 1 mM ouabain. The high concentration was necessary to completely inhibit the pump in this species (Sweadner, 1989). The conditions used for these measurements were chosen to maximize the pump activity: > 50 mM Na in the intracellular solution, 5 mM K in the extracellular solution, and 10 mM MgATP together with nominally zero ADP in the cytoplasm. Current tracings in the presence and absence of ouabain are shown in Fig. 2 A. The effect was usually complete within 90 s after initiating the solution change, and was reversed within 2-4 min after removing ouabain from the superfusate. The I-V curves before and after ouabain are shown in Fig. 2 B, together with the difference curve representing the pump current ($I_{\text{pump}}$). The pump current is always positive (outward) under the conditions of our experiments, and shows only a modest voltage dependence over the voltage range shown here.

A composite $I_{\text{pump}}$-V curve is shown in Fig. 3. This plot combines different pulse protocols in which the cell voltage ranged from +80 to -100 mV. Currents have been normalized to values at zero membrane potential. Over this larger range a significant voltage dependence of the pump current can be discerned. At positive potentials the current appears to saturate. At negative potentials the current becomes progressively smaller, and at $V_m = -100$ mV it declines to ~40% of the value at $V_m = 0$. There is a measurable pump conductance or slope of the $I_{\text{pump}}$-V relationship over the physiological range of membrane voltages (-40 to -80 mV).

To confirm that the ouabain-sensitive currents were indeed reflecting the pump current, we studied the effect of ouabain in the absence of extracellular K. One such experiment is shown in Fig. 4. Removal of K resulted in changes in current similar to
those observed with ouabain. Furthermore, addition of ouabain in zero K solution had no further effect on the electrical properties of the membrane. Since removal of K is expected to inhibit the pump, these data are consistent with the assumption that the major effect of ouabain is also to stop the pump. This result is representative of three different experiments.

A rough dose–response relationship to ouabain is shown in Fig. 5. Since 1 and 3 mM ouabain produced virtually the same current change, these were considered to cause maximal inhibition. Lower concentrations of 0.1 and 0.3 mM ouabain inhibited 41 and 67% of the total pump current, respectively. These data are consistent with a single site of inhibition for the glycoside with an apparent inhibition constant of 0.15 mM.

We next compared the Na channel currents ($I_{Na}$) for CCTs from four groups of rats: control animals on a normal diet, animals on a low-Na diet, animals on a normal diet infused with aldosterone, and animals infused with aldosterone + amiloride. The last three groups were maintained under the stated conditions for 7–14 d. Mean levels of plasma aldosterone measured under these conditions are given in Table I. Aldosterone in control animals was 20 ng/dl. Na depletion for 1–2 wk raised these levels to an average of 1,200 ng/dl. Values obtained with infusion of aldosterone were in the range of 100–300 ng/dl, depending on the concentration of the steroid in the osmotic minipumps.

Fig. 6 shows the values of $I_{Na}$ measured at −60 mV. There was no detectable amiloride-sensitive current in control animals. Those on a low-Na diet and those receiving aldosterone had similar values of $I_{Na}$. From this it appears that elevation of plasma aldosterone was sufficient to induce the channel activity of the Na-depleted animals, since plasma aldosterone was in fact lower in the infused animals than in those that were Na depleted. Infusing the rats with a fivefold larger dose of aldosterone resulted in a similar mean value $I_{Na}$ (195 ± 52 vs. 229 ± 61 pA). The group receiving aldosterone + amiloride also had a similar mean value of $I_{Na}$ (316 ± 61 pA). Infusion of amiloride alone increased both plasma aldosterone and $I_{Na}$ to a small extent (Table I, Fig. 6).

The changes in pump currents ($I_{pump}$) showed a strikingly different pattern (Fig. 6). Mean values for $I_{pump}$ were not different in control and Na-depleted animals. In the aldosterone-infused animals, however, $I_{pump}$ was increased fourfold over the other groups. Increasing the dose of aldosterone fivefold did not increase $I_{pump}$ further.
(132 ± 14 vs. 146 ± 13 pA). However, coinfusion of amiloride at doses designed to achieve concentrations of ~10 μM in the lumen of the CCT (see Materials and Methods) diminished the aldosterone-dependent increase in $I_{\text{pump}}$. The increase in $I_{\text{pump}}$ in the presence of amiloride was 22 pA/cell, while in the absence of the blocker it was 103 pA. Thus, amiloride appeared to block ~80% of the stimulation of pump activity. To see if amiloride also diminished the basal pump activity, we infused amiloride by itself. In this case $I_{\text{pump}}$ was similar to that measured in the presence of high aldosterone + amiloride. If anything, the effect of amiloride alone was a stimulation of $I_{\text{pump}}$. However, the interpretation of this increase in $I_{\text{pump}}$ is complicated since plasma aldosterone also increased under these conditions (Table I).

As shown in Table II, there were no striking changes in the measured cell capacitance during any of these maneuvers. We had previously reported that capacitance was lower in controls vs. Na-depleted animals (Frindt et al., 1990), but this finding was not reproduced in this study. The cells of the aldosterone-infused animals appeared to be larger under the microscope, but the apparent surface as measured by capacitance was only 25% larger than controls, and the increase was not statistically significant.

**DISCUSSION**

**Regulation of Na Channels in the CCT**

In previous series of experiments we used a low-Na diet to induce Na channel activity in the rat CCT (Palmer and Frindt, 1986; Frindt et al., 1990). We had assumed that the mediator of the increase in activity was aldosterone, although this question was not addressed directly. Here we have shown, using the amiloride-sensitive, whole-cell current as an index, that comparable induction of $I_{\text{Na}}$ can be achieved by either Na deprivation or infusion of aldosterone. This supports the assumption that aldosterone is the major, if not the only factor involved in regulating the channels in vivo.
during salt restriction. Fig. 6 shows that similar degrees of stimulation of $I_{Na}$ were achieved with aldosterone concentrations ranging from 110 to 1,200 ng/dl. Below 100 ng/dl, $I_{Na}$ diminishes sharply. Thus, although very high aldosterone levels can be achieved using a physiological maneuver, the range over which Na channels can be regulated in the steady state is relatively narrow, from ~20 to ~100 ng/dl (0.5–3 nM). In the companion paper (Pacha et al., 1993) we described a relationship between single channel activity and aldosterone concentration by a binding isotherm having a $K_d$ of 280 ng/dl. The data on which this was based, however, were also consistent with the idea that channel activity is a steeper function of aldosterone levels, as there was no significant change in activity between 400 and 1,500 ng/dl. The whole-cell current data support the idea of such a steep relationship. To define the relationship more precisely we will need to make more measurements with steady-state hormone concentrations in the range of 20–100 ng/dl.

**Figure 6.** Channel currents and pump currents in different mineralocorticoid states. Channel current was measured as the current inhibited by $10^{-5}$ M amiloride at a cell potential of $-60$ mV. Pump current was measured as the current inhibited by $10^{-3}$ M ouabain at a cell potential of 0 mV. Open bars represent control rats (no channel currents were detected). Filled bars represent rats on a low-Na diet for 1–2 wk. Wavy bars represent rats infused with aldosterone (100 μg/kg body wt·d). Lightly striped bars represent rats infused with aldosterone + amiloride as described in the text. Heavily striped bars represent rats infused with amiloride alone. Data represent means ± SEM for 17–23 determinations. At least three animals were studied under each condition.

The average whole-cell, amiloride-sensitive conductance under Na-depleted or aldosterone-treated conditions was ~ 4 nS/cell. This figure is slightly lower than that reported earlier for studies at room temperature (Frindt et al., 1990). Assuming an apical cell surface area of 150 μm$^2$ and a patch area of 2 μm$^2$, a single channel conductance of 9 pS at 37°C and a $P_o$ of 0.5, we would predict that each patch would have 12 active channels. This is higher than the observed figure of around five per patch (Pácha et al., 1993). Two factors are likely to contribute significantly to this discrepancy. First, the true apical surface area is probably amplified by microscopic infolding. If apical area were one-tenth of the total, then based on a cell capacitance of 36 pF the true apical surface area would be as much as 360 μm$^2$. Second, in the intact cell the channels are subject to feedback regulation (Frindt, Silver, Windhager, and Palmer, 1993; Silver, Frindt, Windhager, and Palmer, 1993). This phenomenon, which is thought to depend on changes in cytoplasmic ions and other factors, could
reduce the number of active channels in cell-attached patches, but may be suppressed by the perfusion of the cell during whole-cell recordings. Given these uncertainties, the whole-cell and single channel results seem to be in reasonably good agreement.

**Na Pump in the CCT**

Na pump currents in the principal cells of the CCT ranged from 35 to 150 pA/cell. Measurements of cell capacitance indicate a pump current density of \( \sim 1 \) pA/pF in Na-depleted rats, 2 pA/pF in control rats, and 3 pA/pF in aldosterone-treated animals. Similar pump current densities have been reported in mammalian heart (Gadsby et al., 1985). The \( I-V \) relationship also resembles that of the heart. Similar \( I-V \) relationships were also described for the amphibian collecting tubule (Horisberger and Giebisch, 1989), although in that study there was little voltage dependence of the pump current in the voltage range of 0 to \(-100 \) mV. The dose–response relationship with respect to ouabain indicates that the apparent \( K_i \) for ouabain is \( \sim 0.15 \) mM. This is typical for the pump isoforms in the rat, which have a low sensitivity to ouabain (Swedner, 1989). This differs from the findings of Doucet and Barlet (1986) that the Na,K-ATPase in the rabbit CCT is a high-affinity form, in contrast to the pumps in the other segments of the rabbit nephron. Our data are consistent with the characterization of the pump in the CCT as a typical "kidney" isoform.

Quantitatively, the pump current density compares well with measurements of Na,K-ATPase activity in this segment. Maximal enzyme activities vary from 750 to 2,000 pmol/mm-h (El Mernissi and Doucet, 1983; Mujais, Chekal, Jones, Hayslett, and Katz, 1985; Barlet-Bas et al., 1990). To compare this with the pump current measurements we make the following assumptions: (a) a stoichiometry of 3Na:2K:1ATP, (b) a lumen diameter of 10 \( \mu \)m, and (c) a luminal surface area of 150 \( \mu \)m\(^2\)/cell. Pump currents of 35 and 140 pA/cell would correspond to turnover rates of 750 and 3,000 pmol/mm-h, respectively. These figures may overestimate turnover rates per millimeter tubule length if the intercalated cell has a lower pump density. This rough calculation suggests that a significant fraction of the pumps whose enzymatic activities can be measured as Na-K-ATPase are probably in the plasma membrane available for transport, and thus are also measureable as \( I_{pump} \) by the whole-cell clamp technique.

**Table 1**

| Condition                        | Cell capacitance | No. of observations |
|----------------------------------|------------------|---------------------|
| Control                          | 38 ± 5           | (13)                |
| Low-Na diet (7–14 d)             | 43 ± 8           | (9)                 |
| Aldosterone infusion, 500 \( \mu \)g/kg · d | 48 ± 5           | (15)                |
| Aldosterone + amiloride infusion. |                  |                     |
| 200 \( \mu \)g/kg · d + 220 \( \mu \)g/d | 56 ± 9           | (6)                 |
| Amiloride infusion, 220 \( \mu \)g/d | 43 ± 6           | (9)                 |
Maximal Na transport rates by the rat CCT in vitro have been reported to be from 3,000 to 9,000 pmol/mm·h in DOCA-treated, ADH-stimulated tubules (Tomita, Pisano, and Knepper, 1985; Rouch, Chen, Troutman, and Schafer, 1991). This corresponds to pump turnover rates of 1,000-3,000 pmol/mm·h and indicates that the pump may be operating at close to maximal capacity under these conditions.

This question can also be addressed by comparing channel activity and pump activity in individual cells. Channel currents at −60-mV membrane potentials were ~300 pA/cell in both Na-depleted and aldosterone-treated rats. Again assuming that the Na efflux through the pump is three times the pump current, the maximal rate of extrusion from the cell is ~450 pA/cell in the aldosterone-treated group. If the apical Na conductance remained this high in the intact cell, where incoming Na would have to be extruded by the pump, the ATPase would have to operate at >50% of capacity. In contrast, for the tubules from Na-depleted rats the pump capacity is well below the maximal rates of Na influx through the channels. In vivo, however, the luminal Na concentration in the CCT may be quite low when the animals are Na depleted, so that rates of Na entry are normally far below those measured in vitro.

**Effects of Aldosterone on the Na Pump**

The literature on the stimulation of the Na pump by aldosterone is massive and has been reviewed recently (Katz, 1990; Marver, 1992; Rossier and Palmer, 1992). There is evidence for both short-term and long-term effects of the steroid and for effects that do or do not involve direct induction of pump synthesis. Furthermore, there is evidence for effects on the pump that require Na entry into the cells (i.e., are blocked by amiloride, which abolishes the effect of the hormone on Na channels; Handler, Preston, Perkins, and Matsumura, 1981; Petty, Kokko, and Marver, 1981; Hayhurst and O’Neil, 1988; Barlet-Bas et al., 1990; Blot-Chabaud et al., 1990), as well as for effects that do not require Na entry (Verrey et al., 1987; Barlet-Bas, Khadouri, Marsi, and Doucet, 1988).

Our data on the pump activity in chronically treated rats are consistent with a simple model for steroid action in which the primary effect of aldosterone is to increase apical membrane permeability. The pump activity is then regulated mainly by the intracellular Na concentration. In Na-depleted animals, Na delivery to the CCT is low, little Na is transported by the segment, and intracellular Na is low. Thus pump activity is minimal, although the apical Na permeability is high. On the other hand, in aldosterone-infused animals, Na delivery to the CCT is high. In conjunction with a high apical Na permeability, there is a high rate of Na transport and a high intracellular Na concentration, leading to stimulation of the pump. This idea has been previously suggested based on data from the rabbit CCT (Petty et al., 1981; Hayhurst and O’Neil, 1988) and from A6 cells (Handler et al., 1981). A number of studies have shown upregulation of the Na pump in a variety of cells, including renal cells, under in vitro conditions in which intracellular Na is expected to increase (Vaughan and Cook, 1972; Rayson and Gupta, 1985; Pressley, Haber, Loeb, Edelman, and Ismail-Beigi, 1986; Wolitzky and Fambrough, 1986). Our data suggest that this may also happen in vivo.

There is evidence that the basolateral membrane area of rat CCT increases with chronic aldosterone treatment in animals on a normal Na diet but not on a low-Na
diet (Wade, Stanton, Field, Kashgarian, and Giebisch, 1990). In this study the increase in basolateral membrane area was nearly twofold after 1 wk. It is therefore surprising that we did not observe a significant increase in the overall membrane capacitance, which should reflect the changes in area. The only significant difference in our protocol was that we used adrenal-intact rats, whereas adrenalectomized animals were used by Wade et al. (1990). This discrepancy remains to be resolved.

The observation that infusion of amiloride partially prevented the increase in pump activity with chronic aldosterone treatment is consistent with the idea that stimulation of the pump is a secondary effect of the hormone. Amiloride would block Na entry into the CCT principal cells, reducing intracellular Na and diminishing the signal for pump stimulation. The relatively small increase in pump current under these conditions could be due to incomplete block of Na entry. Our results are similar to those of Petty et al. (1981), who found that Na,K-ATPase activity was stimulated by acute administration of aldosterone (DOCA) to rabbits in vivo, and that this effect was blocked by codeadministration of amiloride.

An alternative interpretation of this effect of amiloride involves the effects of high concentrations of the drug on other transporters such as the Na-H exchanger or the Na pump itself, or on protein synthesis (Doucet and Barlet-Bas, 1989). With chronic infusion of amiloride, however, we could estimate the steady-state concentration of amiloride in the tubular fluid to be ~ 10 μM. Concentrations were presumably lower in plasma. These concentrations are low compared with those required to block Na-H exchange, Na-Ca exchange, and the Na pump (Kleyman and Cragoe, 1988). Effects on protein synthesis appear to be unlikely since the induction of Na channel activity by aldosterone, which also requires protein synthesis, was not affected.

However, these results need to be reconciled with other observations suggesting a direct effect of aldosterone in the regulation of Na pump expression. Verrey et al. (1987, 1989) have demonstrated, in amphibian epithelia, that the Na,K-ATPase gene is activated by aldosterone. Although the increase in protein synthesis is delayed relative to the increase in Na transport, it is mediated by a Na-independent mechanism. If such a mechanism occurs in the CCT, we would expect an increase in the number of pumps in Na-depleted animals. Species differences are possible. It is also conceivable, however, that newly synthesized pumps are not functional unless intracellular Na is high. Such a model has been suggested by Barlet-Bas et al. (1990) and Blot-Chabaud et al. (1990), who presented evidence for a latent pool of pumps in the CCT which is activated by high intracellular Na and dependent on aldosterone. If such a mechanism were to reconcile the two sets of data, we would have to presume that the high intracellular Na (50 mM) achieved by dialyzing the cells during the whole-cell clamp procedure does not activate the latent pumps, perhaps because the time of exposure to high Na is too short (usually < 5 min).

Alternatively, it is possible that the residual stimulation of pump current observed with infusion of aldosterone + amiloride reflects the effect of direct stimulation of pump synthesis by aldosterone. In this scenario, there could be parallel interdependent effects of the hormone itself and of increased intracellular Na on pump synthesis. The fact that no pump stimulation was observed in the animals on a low-Na diet, where aldosterone levels were quite high, argues against this interpretation.

Barlet-Bas et al. (1988) have shown that aldosterone can directly increase Na,K-
ATPase activity in vitro in rat CCTs by a Na-independent mechanism. This observation is more difficult to reconcile with our results. It is possible, however, that such a short-term stimulation of the enzyme is not sustained in the absence of elevated intracellular Na. This scenario was suggested by O'Neil and Hayhurst (1985), who found that Na,K-ATPase activity in rabbit CCT was elevated when the animals were on a low-Na diet but declined to control levels over a period of 2 wk. In any case the rat CCT may be a good model for studying the effects of Na delivery in vivo on the modulation and biosynthesis of pumps and other transport proteins.

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