Regulation of Apical K and Na Channels and Na/K Pumps in Rat Cortical Collecting Tubule by Dietary K

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ABSTRACT The patch-clamp technique was used to study the properties and the density of conducting K and Na channels in the apical membrane of rat cortical collecting tubule. The predominant K channel observed in cell-attached patches (SK channels) had an outward single-channel conductance (with LiCl in the pipette) of 10 pS. The inward conductance (with KCl in the pipette) was 42 pS. The channel had a high open probability that increased with depolarization. Kinetic analysis indicated the presence of a single open state and two closed states. Increasing K intake by maintaining animals on a high K diet for 12–16 d increased the number of SK channels per patch by threefold (0.7–2.0/patch) over control levels. In addition, conducting Na-selective channels, which were not observed in control animals, were seen at low density (0.5/patch). These channels had properties similar to those observed when the animals were on a low Na diet, except that the mean open probability (0.84) was higher. In other experiments, the whole-cell patch clamp technique was used to measure Na channel activity (as amiloride-sensitive current, I_{Na}) and Na pump activity (as ouabain-sensitive current, I_{pump}). In animals on a high K diet, I_{Na} was greater than in controls but much less than in rats on a low Na diet. I_{pump} was greater after K loading than in controls or Na-depleted animals. These K diet-dependent effects were not accompanied by a significant increase in plasma aldosterone concentrations. To further investigate the relationship between K channel activity and mineralocorticoids, rats were maintained on a low Na diet to increase endogenous aldosterone secretion. Under these conditions, no increase in SK channel density was observed, although there was a large increase in the number of Na channels (to 2.7/patch). Aldosterone was also administered exogenously through osmotic minipumps. As with the low Na diet, there was no change in the density of conducting SK channels, although Na channel activity was induced. These results suggest that SK channels, Na channels and Na/K pumps are regulated during changes in K intake by factors other than aldosterone.

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INTRODUCTION

The mammalian cortical collecting tubule (CCT) can secrete K into the urine by a system which involves uptake of K from the interstitial fluid by the Na/K pump and exit into the urine through ion channels (Giebisch and Field, 1989). It is thought that the apical membrane step is largely mediated by low-conductance K-selective channels which are mildly inwardly rectifying and which have a high open probability at the resting potential of the cell (Frindt and Palmer, 1989; Wang, Schwab, and Giebisch, 1990). These channels are stimulated by protein kinase A and inhibited by cytoplasmic acidification, ATP, and protein kinase C (Wang et al., 1990; Wang and Giebisch, 1991a, b).

Maintaining animals on a high K diet for days to weeks increases the ability of the kidney to secrete K (Giebisch and Field, 1989; Wright and Giebisch, 1992). One of the factors which has been implicated in the control of K secretion is aldosterone. This mineralocorticoid is secreted in response to elevated plasma K levels, and has been shown to increase K secretion, particularly in the CCT, which is a major site of K secretion (Wright and Giebisch, 1992). Indeed, isolated, perfused CCT's taken from mineralocorticoid-treated rabbits (Schwartz and Burg, 1978) and rats (Tomita, Pisano, and Knepper, 1985) secrete K in vitro at rates higher than those from control animals.

The actions of aldosterone on the CCT are complex, however. One well documented effect of the hormone is to elevate Na transport (Schwartz and Burg, 1978; Tomita et al., 1985; Reif, Troutman, and Schafer, 1986), through increases in the density of conducting apical Na channels (Pácha, Frindt, Antonian, Silver, and Palmer, 1993). Na entry into the cells through these channels will depolarize the apical plasma membrane, increasing the driving force for K secretion. Microelectrode studies of rat CCT were consistent with the idea that this increase in driving force could account at least in part for the increased K secretion in response to mineralocorticoids (Schafer, Troutman, and Schlatter, 1990). On the other hand, in rabbit CCT, treatment with mineralocorticoids increased the Ba-sensitive apical membrane K conductance, although this effect was delayed relative to that on the Na conductance (Sanson and O'Neil, 1985).

At the single-channel level, Wang et al. (1990) found that when rats were maintained on a high K diet, the densities of SK channels in cell-attached patches of the apical membrane of the CCT increased. This was consistent with a role of aldosterone in regulating channel density. On the other hand, Frindt and Palmer (1989) reported that rats on a low Na diet, in which aldosterone secretion is also enhanced, did not appear to have an increased number of SK channels. The studies reported here were designed to resolve these issues, in particular to evaluate the correlation between elevated mineralocorticoid status and SK channel activity in the rat CCT.

MATERIALS AND METHODS

Biological Preparations

Sprague-Dawley rats of either sex (100–150 g) raised free of viral infections (Charles River Laboratories, Kingston, NY) were fed either a normal rat chow (Purina Formulab 5008; Na
content 2.8 g/kg, K content 11 g/kg), a high K diet (No. TD76448, Na content 5 g/kg, K content 100 g/kg, Harlan-Teklad, Madison, WI), a matched diet with a normal K content (Na content 5 g/kg, K content 12 g/kg, Harlan-Teklad), or a low Na diet (No. 902902; Na content 3.8 mg/kg, K content 8.6 g/kg, ICN, Cleveland, OH). In some experiments, the animals were implanted subcutaneously with osmotic minipumps (model 2002, Alzet 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 25–75 μg/100 g body wt/d. In one series of experiments, rats underwent bilateral adrenalectomy. These animals were implanted with minipumps and given maintenance doses of a glucocorticoid (corticosterone, 500 μg/100 g body wt/d) and a mineralocorticoid (aldosterone 3.6 μg/100 g body wt/d). In five adrenalectomized animals in which the plasma aldosterone was analyzed the level was 12 ± 6 ng/dl. This was similar to the value expected for the rate of aldosterone infusion (14 ng/dl). In a sixth animal, which was on high K, the measured concentration was 236 ng/dl. We cannot at this point explain this value, because it was much higher than that observed even with adrenal-intact animals on high K. The channel densities in this animal were similar to those from the others with low aldosterone.

Animals were killed by cervical dislocation, the kidneys removed, and the CCT dissected free and opened manually to expose the luminal surface. The split tubules were attached to a small plastic rectangle coated with Cell-Tak (Collaborative Research, Inc., Bedford, MA) and placed in a perfusion chamber mounted on an inverted microscope. Measurements were carried out at 37°C. Principal cells of the tubule were identified visually as described previously (Pácha, Frindt, Sackin, and Palmer, 1991).

**Solutions**

Tubules were superfused with solution consisting of (in millimolar): 140 NaCl, 5 KCl, 2 CaCl₂, 1 MgCl₂, 2 glucose and 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES) adjusted to pH 7.4 with NaOH. The patch-clamp pipettes were filled with solution containing (in millimolar): 140 LiCl, 3 MgCl₂ and 10 HEPES, adjusted to pH 7.5 with NaOH. This solution was used rather than a Na-containing solution because the single-channel currents are larger when Li is the conducted ion. This facilitates the analysis of the patch-clamp records. In some experiments, 140 mM KCl was substituted for the LiCl.

**Electrical**

Basic patch-clamp methods were as described previously (Palmer and Frindt, 1988; Pácha et al., 1993). Recording of currents and analysis of data were carried out with 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. Construction of open and closed time histograms and fitting with exponential functions were carried out according to the method of Sigworth and Sine (1987) using the TAC program (Instrutech). The number of channels in the patch (N) was determined from the number of discrete current levels observed. This may in some cases underestimate N particularly when N is large. Because this will cause the effects of maneuvers which increase channel density to be underestimated, if anything, this simple method was considered to be adequate. As shown previously, counting channels by fitting cumulative times at each level to a binomial distribution gave similar results in most cases (Palmer and Frindt, 1988). Whole-cell recordings were as described previously (Frindt, Sackin, and Palmer, 1990; Palmer, Antonian, and Frindt, 1993). Whole-cell currents through Na channels were measured as the currents blocked by 10 μM amiloride. Na pump currents were measured as the currents blocked by 1 mM ouabain.
Plasma Steroids

Corticosterone and aldosterone were measured by radioimmunoassay as described in a previous publication (Pacha et al., 1993). Plasma samples were obtained from rats just after sacrifice.

RESULTS

Characteristics of SK Channels

In most experiments the SK channels were recognized from the properties of the outward currents that they carry with LiCl in the pipette and normal NaCl solution in the bath. These solutions were chosen in order to facilitate the recognition of both Na and SK-mediated currents in the same patch. The conductance of the Na channels to Li is higher than that to Na (Palmer and Frindt, 1988). Typical results with a cell-attached patch in which there was a single SK channel are shown in Fig. 1 A. A small outward current can be observed in the absence of an applied potential; depolarizing the membrane patch increased the size of the current. The single-channel conductance of this patch was 7 pS. The average conductance in five patches was $10 \pm 3$ pS (mean ± SD). The current-voltage relationship for these patches is shown in Fig. 1 B. Under these conditions we were never able to observe inward currents through the SK channels, presumably because the Li permeability is very low.

As reported in previous studies (Frindt and Palmer, 1989) the open probability was high at all voltages. There was, however, in all patches, a tendency for the open probability to increase with depolarization. In the patch illustrated in Fig. 1 A, $P_0$ was 0.81 at a pipette potential ($V_{\text{pipette}}$) of zero, and increased to 0.97 at $V_{\text{pipette}} = -80$ mV. The average values of $P_0$ as a function of voltage for five patches containing a single channel which were studied under these conditions are shown in Fig. 1 C.

A kinetic analysis for the patch in Fig. 1 A is shown in Fig. 2. The distribution of open-time intervals of the channel can be well described by a single exponential with a time constant of 53 ms at $V_{\text{pipette}} = -60$ mV. The closed-time histograms are well described by two exponentials with time constants of 1.0 ms for the short closed times and 34 ms for the long closures. The effect of voltage on the kinetic properties of the channels was not studied in detail because of the low signal-to-noise ratio when $V_{\text{pipette}}$ was greater than -40. The one consistent trend which could be discerned was a decrease in the number and duration of long closed events when the patch was depolarized.

The kinetics of the SK channel were further studied in cell-attached patches with 140 mM KCl in the patch-clamp pipette. In this case, the channels carried inward currents at the resting potential, i.e., $V_{\text{pipette}} = 0$. A typical record of such a patch with a single SK channel is shown in Fig. 3 A. The currents were in general much larger, reflecting the greater conductance of the channels for inward K current. For five patches similar to that of Fig. 3 A, the inward conductance measured between $V_{\text{pipette}} = -40$ and +60 mV was $42 \pm 1$ pS. The average $i$-$V$ relationships for these five patches is shown in Fig. 3 B. As with the outward currents, the open probability of the channel decreased slightly but consistently with hyperpolarization of the
FIGURE 1. SK channels in cell-attached patches with LiCl in the pipette. (A) Traces from a typical experiment in which $V_{\text{pipette}}$ was changed from 0 to $-100 \text{ mV}$. There was a single SK channel in the patch. The horizontal lines to the right represent the current levels at which the channel is closed. Traces were sampled at 5 kHz and filtered at 1 kHz. The CCT was from a rat on a low Na diet. (B) Single-channel current as a function of voltage. Data are from five patches similar to that in A, and are plotted as means ± SEM. (C) Open probability vs voltage for five patches similar to that in A. Data are plotted as means ± SEM.
membrane. The average $P_0-V$ relationship for the five patches discussed above is shown in Fig. 3 C.

The kinetics of the channel could be more conveniently studied when the current was inward, due to the larger current transitions and high signal-to-noise ratio. The general pattern is similar to that for outward currents. Open times were distributed with a single exponential (Fig. 4 A). The closed time distribution could be described with two exponentials (Fig. 4 A). The open times decreased with hyperpolarization, and increased with depolarization of the membrane (Fig. 4 B). The shorter closed time showed no consistent voltage dependence. The longer closed time had a biphasic voltage-dependence and was maximum at $V_{\text{pipette}} = 20$ mV. The number of long closures was estimated from the area under the curve describing the long time-constant distribution. The number of long closures per second increased as the membrane potential was made more negative (Fig. 4 C). Thus, the effect of voltage on open probability is accounted for by several factors. As in the case of the analysis of outward currents, the number of long closures, as well as their durations, account in part for the increased $P_0$ with depolarization. The voltage dependence of the mean open time also contributes to the effect of voltage on $P_0$.

**Characteristics of Na Channels**

Na channels were observed in cell-attached patches of tubules from rats on a high-K diet as well as from those on a low-Na diet. A typical record from an animal on a high-K diet is shown in Fig. 5. These channels mediated inward currents at all voltages studied. The mean $i-V$ relationship from five patches with one or two channels is shown in Fig. 5 B. The slope conductance was 13 pS. It is also evident
FIGURE 3. SK channels in cell-attached patches with KCl in the pipette. (A) Traces from a typical experiment in which the pipette voltage was changed from −40 to +80 mV. There was a single SK channel in the patch. Horizontal lines to the right of each trace represent the current levels at which the channel is closed. Traces were sampled at 5 kHz and filtered at 1 kHz. The CCT was from a rat on a high K diet. (B) Single-channel current as a function of voltage. Data are from five patches similar to that in A, and are plotted as means ± SEM. (C) Open probability vs voltage for five patches similar to that in A. Data are plotted as means ± SEM.
FIGURE 4. Kinetics of inward currents through SK channels. (A) Open-time and closed-time histograms for the experiment shown in Fig. 3A. $V_{	ext{pipette}}$ was +80 mV. The open-time distribution was fitted with a single exponential with a time constant of 6.3 ms. The closed-time distribution was fitted with two exponentials with time constants of 0.34 and 17 ms. 93% of the events were in the short time-constant distribution. There are 3,235 events in each distribution. (B) Open-time ($t_{\text{open}}$) and short closed-time ($t_{\text{closed}1}$) constants for five experiments similar to that in A as a function of $-V_{\text{pipette}}$. Data are plotted as means ± SEM. (C) Long closed-time constants ($t_{\text{closed}2}$) and the mean number of closed events/s (# long closed) for five experiments similar to that in A. Data are plotted as means ± SEM.
from the figure that the open probability of the channel was high and the kinetics were slow at all voltages. Mean values for these parameters for the five patches are given in Table I. In most patches they were measured at only one voltage. Detailed kinetic analysis was not attempted due to the small number of open and closed events recorded for these channels.

**Figure 5.** Na channels in a CCT from a rat on a high K diet. (A) Traces from a typical experiment in which the pipette voltage was changed from -40 to +60 mV. There was a single Na channel in the patch. At negative pipette voltages, smaller current transitions attributable to an SK channel can also be seen. Horizontal lines to the right of each trace represent the current levels at which the channel is closed. Traces were sampled at 2 kHz and filtered at 100 Hz. (B) Single-channel current as a function of voltage. Data are from six patches similar to that in A, and are plotted as means ± SEM.

**Effects of a High-K Diet**

Rats were put on a high-K diet for 12–16 d as described by Wang et al. (1990). In CCT’s isolated from these animals 89% (39/44) of patches contained at least one SK channel. In those isolated from control animals 28% (11/40) of patches contained an
Table 1

Characteristics of Na Channels from Rat on a High K Diet

| Characteristic | Mean ± SEM | Range       |
|----------------|------------|-------------|
| $P_o$          | 0.84 ± 0.10| 0.65 - 0.90 |
| $\tau_{\text{open}}$ | 1,080 ± 440 ms | 317 - 1,620 |
| $\tau_{\text{closed}}$ | 176 ± 63 ms | 113 - 267   |
| $g$            | 13 ± 1 pS  | 11 - 14     |

SK channel. These results are similar to those reported previously (Wang et al., 1990). This effect was further quantified by estimating the number of channels ($N$) per patch. This estimate was obtained by examining the number of discrete current levels. In patches containing many channels, this will underestimate $N$. Because patches with more than three channels were more common when the animals were on a high K diet, the effect of the diet on $N$ will, if anything, be underestimated. As shown in Fig. 6, the average value of $N$ was 0.70 on the normal or control diet, compared to 1.95 on the high-K diet.

A second effect of the high-K diet was to induce the appearance of conducting Na channels. In animals on the control diet none of the patches (0/29) contained detectable channels. In animals on the high-K diet, 6/24 patches had Na channel activity. The average value of $N$ in these patches was 1.0 (Fig. 6). This is an approximate number since several of these patches contained six or more channels, and $N$ may have been underestimated.

A third effect of the high-K diet was to increase the activity of the Na/K pump measured as a ouabain-sensitive current under conditions of whole-cell clamp (Palmer et al., 1993). As shown in Fig. 7, the pump current, measured at a cell potential of 0, was 100 ± 6 pA/cell. This is significantly larger than the value measured in controls of 25 ± 5 pA/cell. In the same cells, the Na channel activity was measured as the amiloride-sensitive current at a cell potential of −60 mV. The mean value in the animals on a high K diet was 57 ± 18 pA/cell, compared to undetectable levels 2 ± 4 pA/cell in controls.

As discussed in the introduction, aldosterone has been postulated as a mediator of the effects of a high K intake on renal function. Indeed, the appearance of

![Figure 6. Mean number of SK and Na channels per patch for rats on a control diet (open bars) and a high-K diet (dark shaded bars), low-Na diet (waved bars) and infused with aldosterone (light shaded bars). No Na channels were observed in rats on a control diet. The data for the controls were from 30 patches (five rats) on normal rat chow and 14 patches (one rat) on the control diet matched to the high K diet. Results of these two groups were similar.](image)
conducting Na channels and the increase in Na pump current are events which are observed when circulating mineralocorticoid levels are increased (Pácha et al., 1993; Palmer et al., 1993). However, measurements of plasma aldosterone concentration after two weeks on a high K diet were $39 \pm 11 \text{ ng/dl}$ compared to $27 \pm 6 \text{ ng/dl}$ in controls. The distribution of these values is shown in Fig. 8. Although two of the values appeared to be somewhat elevated, there was no consistent or statistically significant increase. There was also no apparent correlation between these levels and the activity of Na channels measured in tubules from the same animals. Levels of corticosterone, the major glucocorticoid in rats, were $37 \pm 11 \mu\text{g/dl}$ in controls and $27 \pm 7 \mu\text{g/dl}$ in animals on the high K diet.

**Effects of a Low Na Diet**

To further examine the relationship between K channel activity and circulating aldosterone, we studied rats placed on a low-Na diet for 7–14 d. We had shown previously that plasma aldosterone levels were greater than 1,000 ng/dl under these conditions (Pácha et al., 1993). As shown in Fig. 6, there was a large induction of conducting Na channels under these conditions, with $N = 2.66$ channels/patch. However, the number of SK channels was not increased above control levels, with $N = 0.5$ channels/patch. This is consistent with previous results which were obtained
Effects of Aldosterone Infusion

Previous studies showed that a low Na diet increased Na channel activity but not that of the Na/K pump, whereas infusion of exogenous aldosterone increased both Na channel and Na/K pump function (Palmer et al., 1993). We therefore examined the effects of aldosterone infusion on SK channel density. Similar results were obtained with animals infused with aldosterone at rates of 25 and 75 μg/100 g/d, and these are pooled and shown in Fig. 6. Again, a substantial induction of active Na channels was observed ($N = 1.65$ channels/patch), but there was no increase in SK channels ($N = 0.48$ channels/patch). Plasma aldosterone levels were 109 ± 13 ng/dl and 238 ± 35 ng/dl in the two groups.

![Figure 9](image)

**Figure 9.** Mean number of SK and Na channels per patch for adrenalectomized rats on a control diet (open bars) and a high-K diet (shaded bars). No Na channels were observed in rats on a control diet.

Effects of Adrenalectomy

Because increases in plasma aldosterone appeared to be neither necessary nor sufficient to increase the SK channel activity, we examined the effects of a high-K diet on the kidneys of animals after a bilateral adrenalectomy and infusion of a low maintenance dose of corticosterone and aldosterone. As shown in Fig. 9, there was no increase in SK channel activity in adrenalectomized animals on a high-K diet compared to those on a control diet. In contrast, there was still an induction of conducting Na channels, although to a somewhat smaller extent than in intact animals. Na channels were observed in 9/43 patches and $N$ was 0.37 channels/patch. No Na channels were observed in 22 patches on tubules from adrenalectomized rats on a control diet.

DISCUSSION

Characteristics of SK Channels

The properties of the SK channels were similar to those previously reported (Frindt and Palmer, 1989; Wang et al., 1990). The inward conductance (40 pS) was higher...
than the outward conductance (10 pS), although these numbers are not strictly comparable in this study because the outward conductance was measured with K on only one side of the membrane, and the inward conductance was measured with K on both sides of the membrane. The open probability was high at all voltages, although we demonstrate here a weak but consistent effect of voltage on $P_0$, which decreases with hyperpolarization.

The kinetics of the SK channel have not previously been studied in detail. Under all conditions that we studied, the open-time distribution was characterized by a single time constant whereas the closed-time distribution indicated the existence of two time constants. This is consistent with there being at least two closed states of the channel. The mean open time decreased with hyperpolarization of the membrane, while the amount of time spent in the long-lived closed state increased. The mean durations of the open states and the predominant shorter closed states are similar to those reported previously (Wang et al., 1990).

### Long-term Regulation of SK Channels

The finding that SK channel density was increased in animals on a high K diet confirms the results of Wang et al. (1990). This effect makes good sense as a homeostatic response, as the increased number of channels will facilitate K secretion into the urine when K intake is increased, helping to maintain overall K balance in the body.

Aldosterone has been implicated in the regulation of K secretion by the CCT (Wright and Giebisch, 1992). Increases in the rate of secretion of aldosterone by the adrenals (Boyd, Palmore, and Mulrow, 1971) and in the circulating levels of the hormone (Stanton and Giebisch, 1982) have been observed when K intake is chronically increased. This presumably reflects a direct stimulation of secretion by the adrenals by high plasma K. Furthermore, increased levels of mineralocorticoids can lead to an enhanced ability of the kidneys to secrete K, especially when plasma K is acutely increased (Wright, Strieder, Fowler, and Giebisch, 1971; Stanton, Klein-Robbenhaar, Wade, and Giebisch, 1985). Finally, studies on rabbit CCT revealed an increase in the Ba-sensitive conductance of the apical membrane when the animals were treated with the mineralocorticoid DOCA (Sansom and O'Neil, 1985).

The results of this study, however, indicate that aldosterone is not the primary factor in the long-term regulation of the density of conducting SK channels. To begin with, the upregulation of the channels was accomplished with little or no increase in circulating aldosterone levels. Furthermore, increasing plasma aldosterone, either by stimulating endogenous secretion with a diet low in Na or by exogenous infusion of the hormone, did not increase SK channel density. Thus, a large increase in circulating aldosterone is neither necessary nor sufficient to augment K channel activity.

Two important qualifications to this conclusion should be emphasized. First, there is strong evidence that large increases in dietary K can increase aldosterone secretion. We presume that the difference between our results and those of Stanton and Giebisch (1982), which demonstrated an increase in circulating aldosterone levels, reflects the degree of K "loading." In the previous studies, the rats were given 100 mM KCl in their drinking water, as well as high K chow. The second point is that
increased plasma aldosterone is expected to stimulate renal K secretion even if the apical K channel density is unchanged. Activation of Na channels will depolarize the apical membrane and increase the driving force for K movement through the existing K channels. In fact, Schafer et al. (1990) have suggested that the increase in K secretion by isolated, perfused CCT's from mineralocorticoid-treated rats can be largely accounted for by an increase in electrical driving force for K movement across the apical membrane.

Our findings are also consistent with previous studies which indicated that stimulation of K secretion can be at least partly dissociated from increased aldosterone. Stanton and co-workers (Stanton, Pan, Deetjen, Guckian, and Giebisch, 1987) found that a chronic high K diet increased the ability of the rat kidney to secrete an acute K load. This adaptation could be observed in adrenalectomized animals, although increased aldosterone levels augmented the effect. Studying isolated, perfused rabbit CCT's, Wingo and co-workers (Wingo, Seldin, Kokko, and Jacobsen, 1982) found that rates of Na reabsorption and of K secretion could be modulated by manipulations of the K content of the diet even after adrenalectomy. Muto, Sansom, and Giebisch (1988) measured increases in apical Na and K conductances in the CCT of adrenalectomized rabbits in response to a high K diet.

Our current hypothesis, which can account for all the data obtained with the rat, is shown in Fig. 10. We suggest that there are two pathways for long-term increases in K transport. The first, which is more important in the mild K loading used in this study, entails (a) an increase in apical membrane K conductance through stimulation of SK channels, (b) a small increase in Na channel activity, and (c) stimulation of the Na/K pump. All of these effects are presumed to be independent of plasma aldosterone. The second pathway does involve increased aldosterone levels. The major effect of the hormone is to stimulate Na channels and an increase in driving force for K movement from cell to urine. The activity of the Na pump will be increased under these conditions, at least in part as an indirect consequence of increased Na entry.
into the cell (Palmer et al., 1993). This second pathway is activated under conditions of more extreme K loads, and can act synergistically with the first.

The factor responsible for mediating the increase in SK channel density is unknown. It could be a second hormone, and the fact that adrenalectomy abolished the effect raises the possibility of another adrenal steroid or of catecholamines. Corticosterone, the major glucocorticoid in the rat, is an unlikely candidate because its plasma levels did not increase on the high-K diet. It is also possible that increased plasma K per se could have a direct influence on the cells of the CCT, as suggested by Wang et al. (1990). In this case, the presence of an intact adrenal gland would exert a permissive effect.

Control of Na Channels

Previous studies of Na transport by isolated perfused tubules (Tomita et al., 1985; Reif et al., 1986), whole-cell currents of principal cells (Frindt et al., 1990; Palmer et al., 1993), and cell-attached membrane patches (Palmer and Frindt, 1986; Pácha et al., 1993) have all failed to detect Na channel activity in CCT's from rats with a normal mineralocorticoid status fed normal rat chow. It was consequently somewhat surprising to find active Na channels in tubules from rats on a high-K diet even in the absence of a significant increase in plasma aldosterone. The presence of the channels was confirmed in both cell-attached patches and with whole-cell clamp, although in both cases the overall channel activity was considerably lower than that observed when the animals were Na depleted or infused with aldosterone. The persistence of the channel activity even in adrenalectomized rats further supports the idea that the channels can be induced by factors other than mineralocorticoids. It is possible that the same (unknown) factors which control K channel density may also contribute to regulation of Na channels.

The single-channel properties of the Na channels observed in K-loaded rats were similar to those previously reported for channels in Na-depleted animals studied under the same in vitro conditions (Frindt, Silver, Windhager, and Palmer 1993). The major difference was an increase in the mean open time and the open probability. The high $P_0$ many reflect the relief of the channels from feedback inhibition secondary to high rates of Na entry into the cells (Frindt et al., 1993; Silver, Frindt, Windhager, and Palmer, 1993). Na entry into the cells from the animals on high K is expected to be lower due to the lower density of conducting channels. Although the tubules were taken from animals with low levels of plasma aldosterone, there was no indication of Na channels with low $P_0$ as has been observed in A6 cells depleted of mineralocorticoids (Kedemey, Kleyman, and Eaton, 1992).

Regulation of the Na/K Pump

Our results also suggest that the Na/K pump in the CCT can be regulated by factors other than aldosterone. In CCT's from rats maintained on a high K diet, pump activity was increased threefold over control levels without a significant change in plasma aldosterone. This is consistent with previous findings that Na/K-ATPase activity increased during K adaptation even in adrenalectomized animals (Silva, Brown, and Epstein, 1977). However, the absolute level of pump current (100 ± 6
pA/cell) was significantly lower than that observed (146 ± 13 pA/cell) when animals were infused with aldosterone (Palmer et al., 1993).

The mechanism underlying this stimulation of the pump by high dietary K is not known. We had previously suggested that the intracellular Na level or load on the pump could mediate long-term changes in pump activity and could account at least in part for the large pump currents observed in the aldosterone-treated animals (Palmer et al., 1993). Because Na channels were also activated by the high K diet, it is possible that this mechanism could also contribute to the stimulation of the pump by K loading. However, we expect that the increase in intracellular Na, like the increase in Na channel activity, will be relatively small under these conditions. It is also conceivable that the same aldosterone-independent factors which affect SK and/or Na channels under these conditions are also involved in upregulating the pump (Fig. 10). We have no data to distinguish these possibilities. It might be possible to test them by treating the K-loaded animals with amiloride to block the increase in cell Na.

Conclusions

The major conclusion of this study is that epithelial Na and K channels can be regulated separately. When animals are Na depleted (but not K loaded), aldosterone

| Condition          | SK channels/patch | Na channel/patch | Aldosterone |
|--------------------|-------------------|------------------|-------------|
| Control            | 0.70 (40)         | 0 (29)           | 27 ± 6      |
| Low-Na diet        | 0.50 (58)         | 2.66 (70)        | > 1,000     |
| +Aldosterone       | 0.48 (54)         | 1.65 (325)       | 174         |
| High-K diet        | 1.96 (44)         | 1.0 (25)         | 39 ± 11     |

levels are high, Na channel density is very high, and SK channel density remains at levels observed in controls. On the other hand, when the rats are K loaded (but not Na depleted) K channel density is increased but Na channel density is much lower (Table II). Thus, the channels can be modulated independently according to the needs of the animal to retain Na and excrete K.

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