Cellular morphology in outer medullary collecting duct: effect of 75% nephrectomy and K+ depletion

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Zalups, Rudolfs K., and David A. Henderson. Cellular morphology in outer medullary collecting duct: effect of 75% nephrectomy and K+ depletion. Am. J. Physiol. 263 (Renal Fluid Electrolyte Physiol. 32): F1119-F1127, 1992.—The present study was designed to determine, in rats, whether 75% nephrectomy and potassium depletion affect the principal and intercalated cells in the outer medullary collecting duct in the same manner as they affect the principal and intercalated cells in the cortical collecting duct. Ten days after a 75% reduction of renal mass, whole animal glomerular filtration rate decreased and the fractional excretion of potassium increased in rats. However, no morphological changes occurred in either the principal or intercalated cells of the outer medullary collecting duct under the same conditions. Further, they noted in intercalated cells of the cortical collecting duct that both the cortical and outer medullary portions of the collecting duct contain principal and intercalated cells found in the outer medullary portion of the collecting duct. Previous findings from the same animals used in the present study show that 75% nephrectomy caused hypertrophic changes in principal cells of the cortical collecting duct, which could be inhibited by potassium depletion induced by the dietary restriction of potassium. The findings also show that the intercalated cells of the cortical collecting duct in 75% nephrectomized rats were unaffected by potassium depletion. On the basis of our findings, it appears there is an absence of hypertrophy in either the principal or intercalated cells in the outer medullary collecting duct under the same conditions. The experiments designed around this secondary aim will provide data to indicate whether there is a similarity in response to 75% nephrectomy between principal cells in cortical collecting ducts and principal cells in outer medullary collecting ducts.

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The present study is actually an extension of a previous study described above (20), in which the effects of 75% nephrectomy and potassium depletion on the morphology of the principal cell in cortical collecting ducts were studied in rats. Samples of inner stripe of the outer medulla containing portions of the outer medullary collecting duct were taken from the animals used in that study, embedded, and stored for future study. The original intent was to apply the same hypothesis to the principal cells in both the cortical and outer medullary collecting duct. The advantage of using these samples of renal tissue is that established responses to 75% nephrectomy and potassium depletion have already been documented in the principal and intercalated cells of the cortical collecting ducts in the remnant kidneys from these animals.

MATERIALS AND METHODS

Animals and Surgical Procedures
The animals used in the present study were the same animals used in the study by Zalups (20). In brief, 21 male Sprague-Dawley rats weighing 250–270 g were used. The animals were divided into three groups. Two groups of animals underwent a 75% reduction of renal mass (n = 15), and the third group underwent a sham operation (n = 6). The operative procedures used on these animals have been outlined in detail previously (20).

Diets and Animal Care
After surgery, six 75% nephrectomized (NPX) rats were placed on a semipurified casein based diet deficient in potassium (0.011% potassium; diet no. 39-304, Ziegler Brothers, Gardners, PA). These animals will be referred as low-K NPX animals. The remaining nine NPX rats and the six sham-operated (SO) rats were placed on the same potassium-deficient diet, except 1.0% potassium chloride was added to it to raise the content of potassium in the diet up to 0.493%. These animals will be referred as normal-K NPX and normal-K SO rats. The animals were fed their respective diets for 10 days after surgery. During the initial 9 days of the feeding trial, the animals were kept in stainless steel cages. For the initial 4 days of the feeding trial, the normal-K NPX and normal-K SO rats were pair fed with respect to the low-K NPX rats. After the initial 4 days of the feeding trial, food was provided ad libitum. During the last 24 h of the study (10th day after surgery), all the animals were placed in plastic metabolic cages so that urine could be collected. During the entire course of this study, all animals were provided water ad libitum.

Balance Studies
The volume of urine excreted in 24 h by the animals used in this study was determined gravimetrically. At the end of the collection period, each animal in the study was anesthetized with pentobarbital sodium (50 mg/kg ip) and then a blood sample was drawn from the inferior vena cava. Subsequently, the left kidney of each animal was fixed in situ by a retrograde perfusion method, which has been described in detail previously (20). Just before fixation, the left kidney was perfused with warm (37°C) buffer (290 mosmol/l), containing 4.3 g/l sodium dihydrogen phosphate, 14.8 g/l disodium monohydrogen phosphate and 1.00 IU/l heparin sodium, at ~120 mmHg pressure to wash out the blood elements from the renal vasculature. After the kidney was cleared of blood, ~60 ml of warmed (37°C) fixative (500 mosmol/l) buffer used to wash out the blood plus 2% (vol/vol) glutaraldehyde was perfused through the left kidney at 120 mmHg pressure. Once the left kidney of each animal was fixed adequately by perfusion, the kidney was removed and a 1-mm midtransverse section of the kidney was obtained. Subsequently, the slice of left kidney was dissected further to obtain 1.0-mm³ blocks from the inner stripe of the outer medulla near the junction of the outer and inner stripes of the outer medulla. These blocks of tissue were further fixed for 3 h in the same fixative and then were washed in three 5-min changes of a 0.1 M s-collidine buffer containing 5.45% (wt/vol) sucrose. The tissues were then postfixed with 1.33% (wt/vol) osmium tetroxide in 0.1 M s-collidine buffer. After the tissues were postfixed, they were washed thoroughly with 0.1 M s-collidine buffer and stained en bloc in 6% (wt/vol) uranyl acetate. Then the blocks were dehydrated in an ascending series of graded ethanol, washed in propylene oxide, and finally embedded in EM bed-812 (Electron Microscopy Sciences, Fort Washington, PA).

Thick and thin sections of the blocks of inner stripe of the outer medulla were obtained using a diamond knife and an AO Ultracut ultramicrotome (AO/Reichert, Buffalo, NY). Thin sections (60–80 nm) were mounted on copper grids and were stained with 6% (wt/vol) aqueous uranyl acetate and 0.3% (wt/vol) lead citrate. Electron microscopic examination of the sections was performed with a Phillips 400 transmission electron microscope. All the blocks were coded before sectioning and microscopy to eliminate any possible bias during the ultrastructural and stereological analysis. Both principal and intercalated cells of outer medullary portions of the collecting duct were examined and photographed. The selection of cells for examination was performed in a random manner. Cells that were sectioned transversely with respect to the basal lamina and that contained two visible and opposing zonulae occludens were examined qualitatively under the electron microscope at...
The cells were later examined quantitatively on electron micrographs printed to a final magnification of ×10,000. Between 15 to 17 cells of each type were evaluated for each animal. Cell and membrane parameters were estimated using standard stereological methods (19) and a specifically designed grid (11) containing semicircular lines spaced 1 cm apart and points spaced 1 cm apart. The semicircular lines on the grid have an isotropic density that eliminates errors arising from anisotropic orientation of renal epithelial membranes. Surface density (S_v; membrane area/cell volume) of both luminal and basolateral membranes was estimated using the equation

$$S_v = \frac{4}{\pi} \times \frac{I_j}{d \times P_T}$$

where $I_j$ is the number of times that each membrane intersects any of the semicircular lines of the test grid, and $P_T$ is the number of test points on the test grid that fall within the boundary of the cell. The symbol $d$ represents the distance between the test points (1 cm) divided by the magnification (×10,000), which is in this case equal to 1.0 μm. In addition, the boundary length ($B$) of both the luminal and basolateral membranes for both principal and intercalated cells was computed using the following equation

$$B = I_j \times P_T$$

The last parameter measured in both cell types was cross-sectional area ($A$), which was computed using the following equation

$$A = d^2 \times P_T$$

The counts for each parameter measured in the 15–17 cells of each type for each animal were averaged to yield means that

### Table 1. Plasma and urinary electrolyte and clearance data

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>$P_{Na^+}$, meq/l</th>
<th>$P_{K^+}$, meq/l</th>
<th>$P_{Cr}$, μmol/l</th>
<th>$V$, ml/24 h·100 g^{-1}</th>
<th>GFR, ml/24 h·100 g^{-1}</th>
<th>$U_{Na^+}$, meq/24 h·100 g^{-1}</th>
<th>$P_{Na^+}$, %</th>
<th>$U_{K^+}$, meq/24 h·100 g^{-1}</th>
<th>$P_{K^+}$, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal-K SO</td>
<td>6</td>
<td>144±3</td>
<td>2.29±0.13</td>
<td>42.3±3.2</td>
<td>7.06±0.32</td>
<td>0.62±0.09</td>
<td>0.40±0.04</td>
<td>0.34±0.05</td>
<td>0.81±0.07</td>
<td>29.5±3.7</td>
</tr>
<tr>
<td>Normal-K NPX</td>
<td>9</td>
<td>144±2</td>
<td>3.64±0.14</td>
<td>85.4±5.2*</td>
<td>9.20±1.29</td>
<td>0.27±0.04*</td>
<td>0.36±0.04</td>
<td>0.73±0.10*</td>
<td>0.96±0.13</td>
<td>67.4±1.8*</td>
</tr>
<tr>
<td>Low-K NPX</td>
<td>6</td>
<td>143±2</td>
<td>2.48±0.24*</td>
<td>79.5±8.6*</td>
<td>12.56±1.44*</td>
<td>0.29±0.02*</td>
<td>0.31±0.02</td>
<td>0.53±0.05</td>
<td>0.07±0.01†</td>
<td>7.48±1.55†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats fed for 10 days. Normal-K SO, sham-operated rats fed a normal-K+ diet; normal-K NPX, 75% nephrectomized rats fed a normal-K+ diet; low-K NPX, 75% nephrectomized rats fed a low-K+ diet; $P_{Na^+}$, plasma [Na+]; $P_{K^+}$, plasma [K+]; $P_{Cr}$, plasma [creatinine]; $V$, rate of urine flow; GFR, glomerular filtration rate; $U_{Na^+}$, absolute excretion of Na+; $F_{Na^+}$, fractional excretion of Na+; $U_{K^+}$, absolute excretion of K+; $F_{K^+}$, fractional excretion of K+. * Significantly different (P < 0.05) from mean for normal-K SO group. † Significantly different (P < 0.05) from mean for normal-K NPX group.

The counts for each parameter measured in the 15–17 cells of each type for each animal were averaged to yield means that

*Fig. 1. Principal cell (A) and intercalated cell (B) in an outer medullary collecting duct of kidney from sham-operated (Sham) rat fed a normal-K diet for 10 days after surgery (×4,894).*
were used to estimate each parameter in the three groups of animals.

Statistical Analysis

All values are means ± SE. Differences between means for each parameter for the three groups of rats were evaluated statistically using the one-way analysis of variance followed by the Dunn’s multiple comparison test (9), which is a post hoc test for planned comparisons. Values for $P < 0.05$ were regarded as statistically significant.

RESULTS

Functional Analysis

The weights of the normal-K SO rats (301 ± 17 g), normal-K NPX rats (299 ± 12 g), and low-K NPX rats (271 ± 5 g) were not significantly different from one another after 10 days of recovery from surgery. However, the concentration of creatinine in plasma was significantly greater and glomerular filtration rate (GFR; estimated by creatinine clearance) was significantly lower in the low-K and normal-K NPX rats, compared with the concentration of creatinine in plasma and GFR in the SO rats (Table 1).

No significant differences in the concentration of sodium in the plasma were detected among the three groups of rats 10 days after surgery (Table 1). Moreover, there was no significant difference in the concentration of potassium in the plasma between the normal-K NPX rats and normal-K SO rats. By contrast, the concentration of potassium in the low-K NPX rats was significantly lower than that in the normal-K NPX rats, indicating that the low-K NPX rats were depleted of potassium.

As was the case with plasma sodium, no changes were observed between the three groups of rats with respect to the absolute excretion of sodium (Table 1). The fractional excretion of sodium, however, was significantly greater in the normal-K NPX rats than in the normal-K SO rats, indicating an effect of 75% nephrectomy.

Absolute excretion of potassium was statistically similar in the normal-K SO and normal-K NPX rats (Table 1). However, fractional excretion of potassium was significantly greater in the normal-K NPX rats than in the normal-K SO rats. In the low-K NPX rats, both the absolute and fractional excretion of potassium was significantly lower than the absolute and fractional excretion of potassium in the normal-K NPX rats and normal-K SO rats.

Structural Analysis

Qualitative observations. At the time of death (10 days after surgery), the remnant kidneys in the NPX rats appeared, on a gross macroscopic level, ~30% greater in size than either of the two kidneys in the normal-K SO rats. This observation indicates that compensatory renal growth occurred in the NPX rats.

Fig. 2. Principal cell (A) and intercalated cell (B) in an outer medullary collecting duct of remnant kidney from a 75% nephrectomized (NPX) rat fed a normal-K diet for 10 days after surgery (X4,580). Note that size of principal cell and intercalated cell is similar in size to corresponding principal cell and intercalated cell in Fig. 1.
For the purpose of comparison, a typical example of a principal cell and intercalated cell in the outer medullary collecting duct in the kidneys of normal-K SO rats, as viewed by electron microscopy, is presented in Fig. 1 (A, principal cell; B, intercalated cell). In general, both types of cells were low cuboidal in shape. The intercalated cells differed from the principal cells structurally in that the intercalated cells possessed a prominent microvillous luminal membrane and generally contained a greater number of mitochondria than the principal cells. In addition, some of these cells contained numerous small vesicles in the apical cytoplasm. The intercalated cells in the outer medullary collecting duct in the normal-K SO rats appeared to be very similar in structure to the type A intercalated cells that are found in the cortical collecting duct of the rat (18).

The principal cells (Fig. 2A) and intercalated cells (Fig. 2B) in the outer medullary collecting ducts of the remnant kidney in the normal-K NPX rats did not differ significantly in morphology from the corresponding principal cells and intercalated cells in the outer medullary collecting ducts of the kidneys in the normal-K SO rats.

Both the principal cells (Fig. 3A) and intercalated cells (Fig. 3B) in the outer medullary collecting ducts in the kidneys of the low-K NPX rats were substantially greater in size than the corresponding principal cells and intercalated cells in the outer medullary collecting ducts in the kidneys of the normal-K NPX or SO rats. In addition to cellular hypertrophy, large vesicles, resembling large multivesicular bodies, containing electron-dense material were found throughout the cytoplasm of the principal cells. Some electron-dense vesicles were also noted in the

Fig. 3. Principal cell (A) and intercalated cell (B) in outer medullary collecting duct of remnant kidney from a NPX rat fed a low-K diet for 10 days after surgery. Note that principal cell and intercalated cell are considerably larger than corresponding principal cell and intercalated cell in Fig. 2. Also note large membrane-bound vesicles containing electron-dense material in principal cell. At higher magnification, these vesicles resemble large multivesicular bodies. Some electron-dense granules or vesicles are also present in intercalated cell (×4,894).
intercalated cells. Moreover, occasional membrane whorls resembling myelin-like figures were present in some intercalated cells. Furthermore, there was a relative decrease or absence of the small apical vesicles in the apical cytoplasm of the intercalated cells.

**Quantitative morphometry.** The average cross-sectional area of principal and intercalated cells in the outer medullary collecting ducts was statistically similar in the normal-K SO and normal-K NPX rats 10 days after surgery (Fig. 4). This finding confirms the qualitative observation that there was no hypertrophy in either the principal or intercalated cells in the outer medullary collecting ducts present in the remnant kidney of normal-K NPX rats. The cross-sectional area of both principal and intercalated cells in the outer medullary collecting ducts of the remnant kidney of the low-K NPX rats, however, was nearly twice that of corresponding principal and intercalated cells in the outer medullary collecting ducts of the remnant kidney of the normal-K NPX rats or the normal kidneys of the normal-K SO rats. This finding also confirms the corresponding qualitative electron microscopic observations that hypertrophy occurred in principal and intercalated cells of outer medullary collecting ducts in the remnant kidney of the low-K NPX rats.

Boundary length (Fig. 5) and surface density (Fig. 6) of the luminal membrane were greater in the intercalated cells than in the principal cells in the outer medullary collecting ducts present in kidneys of the normal-K SO rats. There was no significant difference in boundary length or surface density of the luminal membrane in either principal or intercalated cells in the outer medullary collecting duct between the normal-K SO rats and the normal-K NPX rats. In the low-K NPX rats, the boundary length, but not the surface density, of the luminal membrane of intercalated cells in the outer medullary collecting ducts was significantly greater than that in the normal-K NPX rats. As a point of information, when the boundary length of any membrane increases in association with cellular hypertrophy in the absence of change in the surface density (which is a measure of membrane area to cellular volume) of that membrane,

the increase in size of the membrane is proportional to the increase in the size of the cell.

The surface density of the basolateral membrane of principal cells in the outer medullary collecting ducts of the normal-K NPX rats was significantly lower than that in the normal-K SO rats (Fig. 7). By contrast, there was no significant difference in the surface density or boundary length of the basolateral membrane of intercalated cells in the outer medullary collecting duct between the normal-K NPX and normal-K SO rats (Figs. 7 and 8). In the low-K NPX rats, the boundary length of the basolateral membrane of intercalated cells in outer medullary collecting ducts was significantly greater than that in the normal-K NPX rats. However, there is no significant difference in the surface density of the luminal membrane of the intercalated cells between these two groups of rats (Fig. 8).

**DISCUSSION**

One of the principal aims of the present study was to determine in rats if the principal cells of the outer medullary collecting duct respond to a 75% reduction of renal
mass in a manner similar to the way the principal cells of cortical collecting duct respond. Despite the fact that the principal cells in the two regions of the collecting duct are similar morphologically, the findings from the present study indicate that there is a major difference in the manner in which the two types of principal cells respond after renal mass has been reduced by 75%, at least under the experimental conditions generated in the present study. It appears that while there is a tremendous overall cellular hypertrophy and an amplification of the basolateral membrane in the principal cells of the cortical collecting duct (20, 21), there is an absence of hypertrophy or any other morphologically discernible response in the principal cells in the outer medullary collecting duct.

This absence of response appears to occur when there is an increase in the fractional excretion of potassium, a consistent finding when there is a significant reduction of renal mass (10, 20, 21). Experimental evidence indicates that this enhanced fractional excretion of potassium is most likely due to enhanced secretion of potassium in the cortical collecting duct because the secretion of potassium in this renal tubular segment has been shown to increase following a significant reduction of renal mass (1, 4, 10). The increased secretion of potassium in the cortical collecting duct is thought to be due to the insertion of membrane-containing Na-K pumps into the basolateral membrane of principal cells (20, 21). The insertion of membrane-containing Na-K pumps also serves as an explanation for the tremendous amplification of the basolateral membrane that occurs in these principal cells.

The absence of a morphological response in the principal cells of the outer medullary collecting duct play a different role from principal cells in the cortical collecting duct in the altered renal potassium homeostasis induced by a significant reduction of renal mass. The absence of a morphological response in the principal cells of the outer medullary collecting duct is consistent with the fact that, as yet, there are no functional data indicating that the secretion of potassium occurs in the outer medullary collecting duct after a significant reduction of renal mass.

There is evidence, from the same animals used in the present study (20), that the amplification of the basolateral membrane that occurs in the principal cells of cortical collecting ducts after renal mass is reduced significantly is due to some factor(s) associated with altered potassium homeostasis, as opposed to the actions of the putative renotropic factor(s). The findings show that the dietary restriction of potassium (which leads to potassium depletion) prevents the amplification of the basolateral membrane and the increase in the overall size of the principal cells in cortical collecting ducts from occurring. Although it is not known which specific factors govern the hypertrophic response in the principal cells of cortical collecting ducts after renal mass is reduced, some recent evidence implicates aldosterone as a major regulator of this response (17). Likely other factors, including the extracellular potassium concentration, directly affect the hypertrophic response, since the surface area of the basolateral membrane of principal cells in the cortical collecting duct can increase in response to a potassium load independent of the effects of aldosterone (7).

Potassium depletion after a significant reduction of renal mass appears to affect the principal cells of the outer medullary collecting duct differently from the corresponding principal cells in the cortical collecting duct. Instead of preventing a hypertrophic response in principal cells, as is the case in the cortical collecting duct (20), potassium depletion induces a hypertrophic response in the principal cells of the outer medullary collecting duct. The hypertrophy is characterized by a general increase in the size of the cell without a specific amplification of either the luminal or basolateral membranes. Similar cellular hypertrophy in the outer medullary collecting duct is well documented in normal animals depleted of potassium (5, 6, 13-15). The electron-dense vesicles seen in the principal cells in the present study are also consistent with potassium depletion in normal animals (14). This indicates that the principal cell in the outer medullary collecting duct responds similarly to potassium depletion.
whether or not there has been a significant reduction in renal mass. Most likely, during potassium depletion, the principal cells of the outer medullary collecting duct participate in the renal conservation of potassium, perhaps by increased reabsorption of potassium.

Hypertrophic changes also appear to occur in the intercalated cells of the outer medullary collecting duct during potassium depletion after renal mass has been reduced. This hypertrophy has also been well documented in normal rats depleted of potassium (5, 15). In both normal and 75% nephrectomized rats, potassium depletion does not appear to affect the intercalated cells in the cortical collecting duct. In the renal cortical collecting duct of the rat, there are type A and type B intercalated cells (18). In the outer medullary collecting duct of the rat, only one type of intercalated cell (8) appears, which is morphologically similar to the type A cell found in the cortical collecting duct. Recent evidence from a study on carbonic anhydrase activity in intercalated cells along the collecting duct indicates that the intercalated cell in the outer medullary portion of the collecting duct is functionally different from the type A cell present in the cortical collecting duct (8). In addition, the data from the present study support the hypothesis that there is a functional heterogeneity between the intercalated cells in the outer medullary collecting duct and those in the cortical collecting duct.

In the cortical collecting duct, the intercalated cells have been implicated in the regulation of acid-base balance by secreting hydrogen ions (16). In one recent study, amplification of the luminal membrane was noted in intercalated cells of cortical collecting ducts in the remnant kidney of 75% nephrectomized rats (21). This response was believed to be the result of the insertion of membrane-containing proton pumps into the luminal membrane to promote the secretion of hydrogen ions to control the metabolic acidosis that can occur after a significant reduction of renal mass. Amplification of the luminal membrane of intercalated cells in the cortical collecting duct is an established response in the kidneys of normal rats in which metabolic acidosis is artificially induced (16). The response of the intercalated cells to 75% nephrectomy, however, appears varied, since no changes in the luminal membrane of intercalated cells in the cortical collecting ducts were noted in the remnant kidney of the 75% nephrectomized rats used in the present study (20). The absence or presence of an amplification of the luminal membrane of intercalated cells in the cortical collecting duct after 75% nephrectomy is probably dependent on the absence or presence or degree of metabolic acidosis.

Metabolic alkalosis can occur during potassium depletion (2), which under some conditions can have an effect of the luminal membrane of intercalated cells in the cortical collecting duct (3). However, there is some evidence that the hypertrophy that occurs in both principal and intercalated cells of the outer medullary collecting duct during potassium depletion is unaffected by metabolic alkalosis (5). Despite the fact that cellular hypertrophy can occur in intercalated cells of the medullary collecting duct in the absence of metabolic alkalosis, some evidence from the present study and a previous study (15) indicates that there is a disappearance of apical vesicles from the apical cytoplasm in these intercalated cells during potassium depletion. It has been demonstrated previously that the disappearance of apical vesicles is due to the vesicles fusing with and becoming incorporated in the luminal membrane. The fact that both principal cells and intercalated cells undergo hypertrophic changes indicates that both principal and intercalated cells in the outer medullary collecting duct of the normal or remnant kidney may play a role in the conservation of potassium during potassium depletion.

In conclusion, the findings in the present study indicate that 75% nephrectomy does not have a morphologically discernible effect on either the principal cells or intercalated cells in the outer medullary collecting duct. By contrast, potassium depletion after renal mass is reduced causes marked hypertrophy in both principal and intercalated cells in this portion of the collecting duct. These responses indicate that there probably is a functional heterogeneity between the principal and intercalated cells in the outer medullary collecting duct and the corresponding principal and intercalated cells in the cortical collecting duct. There clearly needs to be more research to determine the role of both principal and intercalated cells of the outer medullary collecting duct in the altered potassium-homeostasis induced by a significant reduction of renal mass and potassium depletion.

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