Intercalated Cell Subtypes in Connecting Tubule and Cortical Collecting Duct of Rat and Mouse

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Abstract. At least two populations of intercalated cells, type A and type B, exist in the connecting tubule (CNT), initial collecting tubule (ICT), and cortical collecting duct (CCD). Type A intercalated cells secrete protons via an apical H⁺-ATPase and reabsorb bicarbonate by a band 3-like Cl⁻/HCO₃⁻ exchanger, AE1, located in the basolateral plasma membrane. Type B intercalated cells secrete bicarbonate by an apical Cl⁻/HCO₃⁻ exchanger that is distinct from AE1 and remains to be identified. They express H⁺-ATPase in the basolateral plasma membrane and in vesicles throughout the cytoplasm. A third type of intercalated cell with apical H⁺-ATPase, but no AE1, has been described in the CNT and CCD of both rat and mouse. The prevalence of the third cell type is not known. The aim of this study was to characterize and quantify intercalated cell subtypes, including the newly described third non A-non B cell, in the CNT, ICT, and CCD of the rat and mouse. A triple immunolabeling procedure was developed in which antibodies to H⁺-ATPase and band 3 protein were used to identify subpopulations of intercalated cells, and segment-specific antibodies were used to identify distal tubule and collecting duct segments. In both rat and mouse, intercalated cells constituted approximately 40% of the cells in the CNT, ICT, and CCD. Type A, type B, and non A-non B intercalated cells were observed in all of the three segments, with type A cells being the most prevalent in both species. In the mouse, however, non A-non B cells constituted more than half of the intercalated cells in the CNT, 39% in the ICT, and 22% in the CCD, compared with 14, 7, and 5%, respectively, in the rat. In contrast, type B intercalated cells accounted for only 8 to 16% of the intercalated cells in the three segments in the mouse compared with 26 to 39% in the rat. It is concluded that striking differences exist in the prevalence and distribution of the different types of intercalated cells in the CNT, ICT, and CCD of rat and mouse. In the rat, the non A-non B cells are fairly rare, whereas in the mouse, they constitute a major fraction of the intercalated cells, primarily at the expense of the type B intercalated cells.

Intercalated cells play a major role in proton and bicarbonate secretion in the collecting duct. They constitute between 30 and 40% of the cells in the connecting tubule (CNT), cortical collecting duct (CCD), and outer medullary collecting duct (OMCD) (1–3).

At least two populations of intercalated cells, type A and type B, exist in both the CNT and the CCD of rat (4–6), mouse (7), and rabbit (8–10). Type A intercalated cells secrete protons via a vacuolar-type H⁺-ATPase that is located in the apical plasma membrane and in apical tubulovesicles (5,7,9,11,12). Bicarbonate is reabsorbed by a band 3-like Cl⁻/HCO₃⁻ exchanger that is located in the basolateral plasma membrane (6–8,13). Type B intercalated cells secrete bicarbonate, which is mediated by an apical Cl⁻/HCO₃⁻ exchanger that is distinct from the band 3-like anion exchanger present in the basolateral plasma membrane of the type A cells (14,15). A vacuolar-type H⁺-ATPase is located in the basolateral plasma membrane and in cytoplasmic vesicles throughout the type B cells (5,7,9,12). Only one type of intercalated cell has been described in the OMCD. It resembles the type A intercalated cell in the CCD.

A third type of intercalated cell, which is ultrastructurally and immunologically distinct from the type A and type B intercalated cells, has been described in the CNT and CCD of both rat (6,16) and mouse (7). This cell has a vacuolar-type H⁺-ATPase in the apical plasma membrane like the type A intercalated cell, but has no basolateral band 3 immunoreactivity (6,7). The function of this nonA-nonB type of intercalated cell is unknown. It has been reported to be rare in the rat (6,16), and has not been observed in the rabbit. However, it constitutes a large proportion of the intercalated cells in the CNT and the initial collecting tubule (ICT) of the mouse (7).

On the basis of immunohistochemical studies using specific antibodies to the vacuolar H⁺-ATPase and band 3 protein, type A and type B intercalated cells were originally reported to constitute 46 and 53%, respectively, of the intercalated cells in...
the renal cortex of the rat, with the remaining 1% belonging to the third or nonA-nonB type (6). No distinction was made between the CNT and the CCD (6). A recent study from the same laboratory determining the prevalence of intercalated cell subtypes in both the CNT and the CCD of the rat found that type A intercalated cells, identified as band 3-positive cells, constituted 54 and 48% of the intercalated cells in the CNT and CCD, respectively (17). However, only 12 and 34% of the intercalated cells in the CNT and CCD exhibited the basolateral or diffuse labeling for H^+-ATPase characteristic of type B cells. The remainder of the intercalated cells were band 3-negative, but had apical or diffuse/apical staining for H^+-ATPase. Cells with distinct apical staining for H^+-ATPase, but no band 3 immunoactivity, which is characteristic of the third or nonA-nonB type of intercalated cell, constituted 2.7 and 1.9% of the intercalated cells in the CNT and the CCD, respectively (17). Studies in which subpopulations of intercalated cells were identified based on the pattern of H^+-ATPase immunofluorescence labeling alone, have reported that cells with the characteristics of type A and type B intercalated cells constituted 66 and 34%, respectively, of the intercalated cells in the cortex (18). Taken together, these observations suggest that a significant proportion of the band 3-negative intercalated cells in the renal cortex of the rat have apical H^+-ATPase and thus belong to a distinct nonA-nonB type of intercalated cell.

In the mouse, all three subtypes of intercalated cells are present in the CNT and CCD (7). Type A intercalated cells identified by band 3 immunolabeling constitute approximately half of the intercalated cells in the cortex (19), or 40 and 60% of the intercalated cells in the CNT and CCD, respectively (7). The band 3-negative intercalated cells include the type B and the nonA-nonB type of intercalated cell, but the relative prevalence of these cells is not known.

The purpose of this study was to characterize and quantify intercalated cell subpopulations in the CNT and CCD of both mouse and rat. A triple immunolabeling procedure was developed in which specific antibodies to H^+-ATPase and band 3 protein were used to identify subpopulations of intercalated cells, and segment-specific antibodies were used to identify the distal convoluted tubule (DCT) and the collecting duct.

**Materials and Methods**

**Animals and Tissue Preservation**

Six normal adult female ICR mice, 10 to 12 wk of age, weighing 20 to 25 g, and six normal adult female Sprague Dawley rats, 10 to 12 wk of age, weighing 200 to 250 g, were anesthetized with an intraperitoneal injection of sodium pentobarbital, 50 mg/kg body wt. The kidneys were preserved for immunohistochemical studies by in vivo perfusion fixation. Mice were perfused through the left ventricle of the heart and rats through the abdominal aorta. The animals were first perfused briefly with phosphate-buffered saline (PBS) to rinse away all blood. The kidneys were subsequently perfused with the fixative solution, periodate lysine-2% paraformaldehyde, for 3 min. The kidneys were removed and cut transversely into 1- to 2-mm thick slices that were immersed in the same fixative overnight at 4°C.

**Antibodies**

Specific rabbit polyclonal immune sera and affinity-purified antibodies were used to identify distinct cell types in the distal tubule and collecting duct. Immune serum against the 70-kD catalytic subunit of the vacuolar H^+-ATPase (kindly provided by Dr. Dennis Stone, University of Texas Southwestern, Dallas, TX), was used to identify intercalated cells. It labels all intercalated cell subtypes in both mouse (7) and rat (20). An antibody to the erythrocyte Cl^-/HCO_3^- exchanger, band 3 protein (kindly provided by Dr. Philip S. Low, Purdue University, West Lafayette, IN), was used to identify type A intercalated cells. It labels the basolateral plasma membrane of type A intercalated cells of both mouse (7) and rat (13,20). DCT cells were identified using immune serum against the thiazide-sensitive Na^+/Cl^- cotransporter (TSC) (kindly provided by Dr. Steven C. Hebert, Vanderbilt University, Nashville, TN). It labels the apical plasma membrane of DCT cells (21). Principal cells in the CCD were identified using an antibody to aquaporin 2 (AQP-2) (kindly provided by Dr. Mark Knepper, National Institutes of Health, Bethesda, MD). It labels the apical plasma membrane of the principal cells (22).

**Immunohistochemistry**

To identify the three populations of intercalated cells and the tubule segments in which they are located, a triple labeling procedure was used (Table 1). Band 3 protein and TSC were labeled simultaneously by a double-labeling technique using a preembedding method. This was followed by labeling for either H^+-ATPase or AQP-2 on serial sections of the same tissue using a postembedding method. In addition, double-labeling experiments were performed in which AQP-2 was labeled using the preembedding method and H^+-ATPase was labeled by the postembedding method.

**Preembedding Method for Band 3 and TSC Immunolabeling**

Sections of periodate lysine-2% paraformaldehyde-fixed tissue were cut transversely through the kidney on a Vibratome at a thickness of 50 μm and processed for immunohistochemistry using an indirect immunoperoxidase method. All sections were washed with 50 mM NH_4Cl in PBS three times for 15 min. Before incubation with the primary antibodies, all tissue sections were incubated for 3 h with PBS containing 1% bovine serum albumin, 0.05% saponin, and 0.2% gelatin (solution A). The tissue sections were then incubated overnight at 4°C in a mixture of rabbit antisera against TSC (1:1000), and band 3 protein (1:400), in PBS containing 1% bovine serum albumin (solution B). Some sections were incubated only with antibodies to AQP-2 (1:1000). After several washes with solution A, the tissue sections were incubated for 2 h in peroxidase-conjugated goat anti-rabbit IgG, Fab fragment (Jackson ImmunoResearch Laboratories), diluted 1:50 in solution B. The tissues were then rinsed, first in solution A and subsequently in 0.05 M tris(hydroxymethyl)aminomaleate (solution C). The tissues were then incubated for 30 min with DAB DAB DAB (solution D).

**Table 1. Triple labeling by immunohistochemistry**

<table>
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<tr>
<th>Protein-Labeled</th>
<th>Section I</th>
<th>Section II</th>
<th>Section III</th>
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<tbody>
<tr>
<td>TSC and band 3</td>
<td>DAB</td>
<td>DAB</td>
<td>DAB</td>
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<tr>
<td>H^+-ATPase</td>
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<td>Vector SG</td>
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<tr>
<td>AQP-2</td>
<td>Vector SG</td>
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^a TSC, thiazide-sensitive Na^+/Cl^- cotransporter; AQP-2, aquaporin 2; DAB, 3,3’-diaminobenzidine.
methylene (Tris) buffer, pH 7.6. For the detection of horseradish peroxidase, the sections were incubated in 0.1% 3,3′-diaminobenzidine in 0.05 M Tris buffer for 5 min, after which 
\[ \text{H}_2\text{O}_2 \] was added to a final concentration of 0.01% and the incubation was continued for 10 min. After washing with 0.05 M Tris buffer three times, the sections were dehydrated in a graded series of ethanol and embedded in Epon 812. From all animals, 50-μm-thick Vibratome sections through the entire kidney were mounted in Epon 812 between polyethylene vinyl sheets.

**Postembedding Method for \( \text{H}^+ \)-ATPase or AQP-2 Immunolabeling**

From the flat-embedded Vibratome sections processed for double immunolabeling of TSC and band 3 protein or single immunolabeling of AQP-2, sections from the cortex were excised and glued onto empty blocks of Epon 812, and consecutive 1.5-μm sections were cut for \( \text{H}^+ \)-ATPase or AQP-2 labeling. The sections were treated for 5 min with a saturated solution of sodium hydroxide to remove the resin. After three brief rinses in absolute ethanol, the sections were hydrated with graded ethanol and rinsed in tap water. The sections were rinsed with PBS, incubated in normal goat serum for 30 min, and subsequently incubated for 2 d at 4°C with antibodies to either \( \text{H}^+ \)-ATPase or AQP-2, Vector SG (Vector Laboratories) was used as the chromogen to produce a gray blue label, which is easily distinguished from the brown label produced by 3,3′-diaminobenzidine in the first immunolocalization procedure for TSC and band 3 protein or single immunolabeling of TSC and AQP-2. For detection of \( \text{H}^+ \)-ATPase and AQP-2, Vector SG (Vector Laboratories) was used as the chromogen to produce a gray blue label, which is easily distinguished from the brown label produced by 3,3′-diaminobenzidine in the first immunolocalization procedure for TSC and band 3 protein or single immunolabeling of TSC and band 3 protein or single immunolabeling of TSC and band 3 protein.

**Quantification of Subtypes of Intercalated Cells**

To quantify the different populations of cells in the DCT, CNT, ICT, and CCD, we used three consecutive 1.5-μm sections mounted on the same slide. The first and third of the sections were used for identification of tubule segments, and the second section was used to count the number of intercalated cell subtypes. The four segments were identified by the following criteria: (1) The DCT is located in the cortical labyrinth and has apical labeling for TSC. (2) The CNT is located in the cortical labyrinth, but is negative for TSC. It exhibits weak apical AQP-2 immunoreactivity that tends to disappear during the etching procedure that is part of the postembedding method. (3) The ICT is located in the cortical labyrinth, and its principal cells have weak apical AQP-2 immunoreactivity. (4) The CCD is located in the medullary ray, and its principal cells have strong apical labeling for AQP-2.

For cell counting, only cells with a distinct nucleus were included. Intercalated cells were subclassified into four different groups based on the pattern of immunohistochemical labeling for band 3 protein (brown color) and \( \text{H}^+ \)-ATPase (gray-blue color) on the second 1.5-μm section: (1) Type A intercalated cells (cells with apical \( \text{H}^+ \)-ATPase and basolateral band 3 immunoreactivity); (2) Type B intercalated cells (cells with basolateral and/or diffuse \( \text{H}^+ \)-ATPase and no band 3 immunoreactivity); (3) Non A-non B type of intercalated cells (cells with apical \( \text{H}^+ \)-ATPase and no band 3 immunoreactivity); and (4) Bipolar type of intercalated cells (cells with distinct apical as well as basolateral \( \text{H}^+ \)-ATPase but no band 3 immunoreactivity). For each animal, between 250 and 1100 cells were counted from the DCT, between 75 and 575 cells were counted from the CNT, between 37 and 650 cells were counted from the CCD, and between 75 and 400 cells were counted from the ICT. The values are expressed as percentages of the total number of cells in the respective segments and presented as mean values ± SD from six animals.

**Results**

*Identification of the DCT, CNT, ICT, and CCD by Immunolabeling for TSC and AQP-2*

The distribution of TSC and AQP-2 immunoreactivity was similar in mice and rats. TSC immunostaining was observed in the apical region of DCT cells, exclusively, and thus served to identify the DCT and distinguish it from the CNT (Figure 1). Cells of the thick ascending limb and macula densa, CNT cells, and intercalated cells were TSC-negative. Apical AQP-2-immunolabeling was present in the CNT and collecting duct (Figure 2); however, the intensity of staining was much greater in the collecting duct than the CNT. In the CNT, AQP-2 immunolabeling was very weak using the preembedding method (Figure 2A), and there was no labeling of CNT cells using the postembedding method. In the ICT, the principal cells exhibited weak AQP-2 immunoreactivity. However, intense immunolabeling for AQP-2 was observed on the apical membrane of the principal cells in the medullary ray portion of the CCD and throughout the remainder of the collecting duct. Thus, the presence and intensity of AQP-2 immunolabeling and the location of the tubules served to distinguish between the CCD, ICT, and CNT. Intercalated cells were negative for AQP-2.

**Distribution of Cells with \( \text{H}^+ \)-ATPase and Band 3 Immunoreactivity**

Subtypes of intercalated cells were easily identified by their pattern of immunostaining for \( \text{H}^+ \)-ATPase and band 3 protein using double- and triple-labeling procedures on 1.5-μm-thick sections from rat and mouse kidneys. Type A intercalated cells, identified by the presence of apical \( \text{H}^+ \)-ATPase and basolateral band 3 immunostaining, constituted a major portion of the intercalated cells in the CNT, ICT, and CCD of both rat (Figure 3) and mouse (Figures 4 through 6), and they were observed also in the terminal part of the distal convoluted tubule (Figure 3, A and B, and Figure 4). The band 3-negative intercalated cells could be subdivided into three distinct cell populations based on the pattern of \( \text{H}^+ \)-ATPase immunostaining. The type B intercalated cells, characterized by basolateral and/or diffuse localization of \( \text{H}^+ \)-ATPase, were frequently observed in the CCD, ICT, and CNT of the rat (Figure 3), but were much less common in the mouse (Figures 5 and 6), where they were observed mainly in the ICT and CNT. The non A-non B type of intercalated cell, with apical \( \text{H}^+ \)-ATPase similar to the type A intercalated cell but no basolateral band 3 immunoreactivity, was the main form of intercalated cell in the CNT of the mouse (Figure 4) and was also frequently observed in the ICT and CCD (Figures 5 and 6). However, these cells were fairly rare in the rat where they were observed mainly in the CNT (Figure 3). They were large cells that often protruded into the tubule lumen. Finally, a bipolar type of intercalated cell, which was characterized by accentuated staining for \( \text{H}^+ \)-ATPase at both
the apical and basolateral pole, was occasionally observed in the CCD, ICT, and CNT of both species (Figures 3D and 5B).

Quantification of Intercalated Cell Subtypes

The percentages of the intercalated cells in rats and mice were similar in the CNT (42% versus 39%), the ICT (43% versus 36%), and the CCD (41% versus 37%), and they were quite rare in the DCT of both species (7% versus 4%). However, striking differences were observed in the prevalence of the different populations of intercalated cells between rat and mouse, as well as in the segmental distribution of these cells.

The percentages of intercalated cell subtypes in the renal cortex of the rat kidney are presented in Table 2. In the DCT, most of the intercalated cells were type A cells (66%) or the non A-nonB type of intercalated cells (26%). Type B intercalated cells were rarely observed in the DCT (6%). In the CNT, about half (57%) of the intercalated cells were type A cells, whereas type B and the nonA-nonB type of intercalated cell constituted 26 and 14% of the intercalated cells, respectively.

In the ICT and CCD, the percentages of the different subpopulations of intercalated cells were similar. The type A cells constituted approximately half of the intercalated cells in each of the two segments (57 and 53%). Type B cells were also prevalent (32 and 39%), whereas non A-non B intercalated cells constituted only 7 and 5%, respectively. The cells with bipolar distribution of H⁺-ATPase were very rare.

The percentages of intercalated cell subtypes in the renal cortex of the mouse kidney are presented in Table 3. In the late part of the DCT, a small number of intercalated cells were intermingled with the TSC-positive DCT cells. Most of these cells belonged to the non A-non B type (51%) or the type A intercalated cells (46%). Type B intercalated cells were extremely rare in the DCT (3%). In the CNT, more than half (57%) of the intercalated cells belonged to the non A-non B population. Type A intercalated cells were also prevalent (34%) whereas type B cells constituted only 8% of the intercalated cells in the CNT. Cells with bipolar distribution of H⁺-ATPase were very rare.

In the ICT, the type A cells and the non A-non B type each constituted 39% of the intercalated cells, whereas 13% belonged to the type B cells. In the CCD, the majority of the intercalated cells were type A cells (50%). The non A-non B cells constituted 22% of the intercalated cells in the CCD and
were mainly observed in the outer portion of the CCD, and 16% were type B cells. Cells with bipolar distribution of H\textsuperscript{+}-ATPase were observed in about 10% of the intercalated cells in the ICT and CCD.

**Discussion**

The results of this study demonstrate the presence of at least three distinct populations of intercalated cells in the CNT and CCD of both rat and mouse: type A intercalated cells with apical H\textsuperscript{+}-ATPase and basolateral band 3 immunoreactivity, type B intercalated cells with basolateral and/or diffuse H\textsuperscript{+}-ATPase and no band 3 immunoreactivity, and a nonA-nonB type of intercalated cell with apical H\textsuperscript{+}-ATPase similar to that observed in the A cell, but no band 3 immunoreactivity. In addition, band 3-negative cells with a bipolar distribution of H\textsuperscript{+}-ATPase were occasionally observed in both rat and mouse.

The demonstration that type A intercalated cells constitute between 50 and 60% of the intercalated cells in both the CCD and CNT of the rat kidney is in agreement with the results of previous studies by other investigators (4,6,17). The prevalence of type B intercalated cells in the CCD is also similar to findings reported previously (4,6,17). The demonstration of a third type of intercalated cell in the CNT confirms the results of earlier studies from our laboratory (16). However, although the non A-non B cells were fairly rare in the rat, they actually constituted a larger proportion (14%) of the band 3-negative intercalated cells in the CNT than anticipated. It was reported previously by Alper and coworkers (6) that less than 1% of the

*Figure 2. Light micrographs of consecutive 1.5-\mu m sections of rat kidney cortex illustrating single immunostaining for aquaporin 2 (AQP-2) (A) and double immunostaining for AQP-2 and H\textsuperscript{+}-ATPase (B) in the connecting tubule (CNT) and cortical collecting duct (CCD). (A) There is intense apical immunostaining for AQP-2 on principal cells in the CCD compared with the weak labeling of the CNT cells. (B) Intercalated cells, type A (open arrow) and type B (arrowheads), are negative for AQP-2. Magnification, \times660.*
Figure 3. Light micrographs of consecutive 1.5-μm sections of rat kidney cortex illustrating double immunostaining for TSC and band 3 protein (A and C), and triple immunostaining for TSC, band 3 protein, and H^+-ATPase (B and D) in the DCT, CNT, and CCD. Four subtypes of intercalated cells, type A (open arrows), type B (arrowhead), non A-non B (arrows), and bipolar cells (double arrows) can be distinguished. Note the absence of band 3 immunoreactivity in type B cells, non A-non B cells, and bipolar cells (A and C). In the proximal tubule, H^+-ATPase is localized in the submicrovillar region (B and D). Magnification, ×1000.
Figure 4. Light micrographs of consecutive 1.5-μm sections of mouse kidney cortex illustrating double immunostaining for TSC and band 3 protein (A), triple immunostaining for TSC, band 3 protein, and H^+-ATPase (B), and triple immunostaining for TSC, band 3 protein, and AQP-2 (C) in the DCT and CNT. Apical TSC immunostaining is present in the DCT. The majority of intercalated cells in the CNT are large non A-non B type intercalated cells (arrows) with strong apical H^+-ATPase labeling and without band 3 protein. Open arrows indicate type A intercalated cells with apical H^+-ATPase and basolateral band 3 protein. After etching, there is no AQP-2 immunoreactivity in the CNT (C). Magnification, ×660.
Figure 5. Light micrographs of consecutive 1.5-μm sections of mouse kidney cortex illustrating double immunostaining for TSC and band 3 protein (A), triple immunostaining for TSC, band 3 protein, and H^+-ATPase (B), and triple immunostaining for TSC, band 3 protein, and AQP-2 (C) in the ICT. Four subtypes of intercalated cells, type A (open arrow), type B (arrowhead), non A-non B type (arrow), and bipolar cells (double arrows) are present in the ICT. Magnification, ×660.
Figure 6. Light micrographs of consecutive 1.5-μm sections of mouse kidney cortex illustrating double immunostaining for TSC and band 3 protein (A), triple immunostaining for TSC, band 3 protein, and H^+^-ATPase (B), and triple immunostaining for TSC, band 3 protein, and AQP-2 (C) in the CCD. Three subtypes of intercalated cells, type A cell (open arrow), type B cell (arrowhead), and non A-non B cell (arrow), are seen in the CCD. Note that principal cells in the CCD are stained for AQP-2 on the apical plasma membrane (C). Magnification, ×660.
intercalated cells in the rat kidney cortex represent a third, band 3-negative form. A subsequent study by Sabolic et al. (17) reported that 2.7% of all intercalated cells in the CNT and 1.9% of the intercalated cells in the CCD had an apical staining for H^+\text{-ATPase}, but no band 3 immunoreactivity, which is characteristic of the non A-non B type of intercalated cells. However, in that study another group of band 3-negative cells were characterized as having apical/diffuse H^+\text{-ATPase} immunostaining. It is quite likely that the cells with apical/diffuse labeling also included non A-non B intercalated cells, which might explain the discrepancy between the results reported by Sabolic et al. (17) and those of the present study, in which approximately 14% of the intercalated cells in the CNT were of the non A-non B type. However, in the medullary ray portion of the CCD, the non A-non B type of intercalated cells constituted only 5% of the intercalated cells. The fact that these cells are fairly rare in the collecting duct of the rat may explain why they have been generally overlooked until recently.

The most striking observation in the present study was the large number of non A-non B type intercalated cells and the sparsity of type B intercalated cells in the mouse kidney. The non A-non B cells were the most common form of intercalated cell in the CNT and they were prevalent also in the ICT. These observations are in agreement with the results of a previous qualitative study of intercalated cells in the mouse kidney by Teng-umnuay and coworkers (7). However, in the present study non A-non B cells were also observed in the medullary ray portion of the CCD, in contrast to the findings reported by Teng-umnuay et al. (7), and band 3-negative intercalated cells were more prevalent in the CCD than reported previously (7). The reason for these discrepancies is not known. However, it should be pointed out that different immunohistochemical techniques were used in the two studies. In the earlier study (7), cell counts were performed on wax sections that were immunostained using a postembedding procedure. In the present study, cell counts were performed on 1-μm plastic sections of tissue labeled using a preembedding procedure that gives much higher resolution.

In the mouse, type B intercalated cells, identified as band 3-negative cells with basolateral and/or diffuse H^+\text{-ATPase} immunostaining, were fairly rare in both the CNT and CCD, suggesting either that these segments of the renal tubule do not play a major role in bicarbonate secretion in the mouse kidney or that other cell types may be involved in bicarbonate secretion in the CCD and CNT of the mouse.

So far the non A-non B intercalated cells have only been described in the rat and the mouse, and it is not known whether these cells exist in other species. In the rabbit, Emmons and Kurtz (23) reported the presence of two functionally distinct forms of bicarbonate-secreting cells. One form, the classic type B intercalated cell, has an apical Cl^-/HCO_3^- exchange, whereas the second form has both an apical and a basolateral Cl^-/HCO_3^- exchanger. However, subsequent studies by Weiner and colleagues (24) provided evidence that most, if not all, type B intercalated cells in the rabbit CCD have both apical and basolateral Cl^-/HCO_3^- exchange, findings that do not support the existence of two forms of bicarbonate-secreting intercalated cells in the rabbit. There is no evidence for the presence of band 3-negative intercalated cells with apical H^+\text{-ATPase} in the CCD or CNT of the rabbit, and cells with those characteristics have not been described in the human kidney. It should be emphasized that double labeling for H^+\text{-ATPase} and band 3 protein is required to establish with certainty whether the non A-non B intercalated cells are present.

The functional role of the non A-non B type of intercalated cell is not known. However, the strong apical H^+\text{-ATPase} immunoreactivity, much of which is in the apical plasma membrane (7), suggests that these cells are involved in proton secretion into the tubule lumen. The mechanism by which base equivalents are extruded from the cells is not known, and it

<table>
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<th>Tubule Segment</th>
<th>A Cells</th>
<th>Non A-Non B Cells</th>
<th>B Cells</th>
<th>Bipolar Cells</th>
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<td>DCT</td>
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*a Numbers are mean values ± SD (n = 6), expressed in percentage of total number of cells in the different segments. DCT, distal convoluted tubule; CNT, connecting tubule; ICT, initial collecting tubule; CCD, cortical collecting duct.
remains to be established if these cells have a basolateral bicarbonate extrusion mechanism. It should be pointed out that an apical H⁺-K⁺-ATPase that secretes hydrogen in exchange for potassium has also been demonstrated in intercalated cells (25). Whether it is expressed in all intercalated cells remains to be established.

Recent studies suggest that several distinct mechanisms or transporters are responsible for bicarbonate extrusion from cells in the collecting duct. Studies by Weiner and colleagues have demonstrated that, in addition to the well-established AE1-mediated Cl⁻/HCO₃⁻ exchange in the type A intercalated cells, basolateral chloride/bicarbonate exchange also occurs in both type B cells (24) and principal cells (10,26). The identity of the transporters involved in these processes is not known. The Cl⁻/HCO₃⁻ exchanger responsible for bicarbonate secretion in the type B intercalated cells also has not been identified. However, it is known that these transporters are structurally and functionally distinct from band 3 protein, the AE1 isoform of the anion exchanger family that is responsible for bicarbonate reabsorption in the type A intercalated cell, because they do not label with antibodies to band 3 protein (6,7,14) and differ in their sensitivity to disulfonic stilbenes (23,24,26). Whether any of these band 3-negative anion exchangers are present in the non A-non B type of intercalated cells is not known.

Recent studies by Alper and colleagues have examined the expression and cellular distribution of AE2, another member of the anion exchanger family, in kidneys of both rat (27) and mouse (28). Basolateral immunostaining for AE2 was observed in the thick ascending limb, distal convoluted tubule, and in both cortical and medullary collecting duct segments. There was no AE2 immunoreactivity in type A intercalated cells. The non A-non B type of intercalated cells were not identified in those studies, and it is not known if AE2 is expressed in these cells.

In summary, our results demonstrate that at least three populations of intercalated cells, type A, type B, and a non A-nonB type, exist in the CCD and CNT of both rat and mouse. Type A and type B intercalated cells are the most common forms in the rat, whereas in the mouse, the non A-non B form constitutes a major fraction of the intercalated cells in the CNT and ICT, mainly at the expense of the type B intercalated cells. The function of the non A-nonB type of intercalated cell is not known, but based on the presence of apical H⁺-ATPase, we propose that it is involved in acid secretion.

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References


