Cell Proliferation in the Loop of Henle in the Developing Rat Kidney

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Abstract. In the developing rat kidney, there is no separation of the medulla into an outer and inner zone. At the time of birth, ascending limbs with immature distal tubule epithelium are present throughout the renal medulla, all loops of Henle resemble the short loop of adult animals, and there are no ascending thin limbs. It was demonstrated previously that immature thick ascending limbs in the renal papilla are transformed into ascending thin limbs by apoptotic deletion of cells and transformation of the remaining cells into a thin squamous epithelium. However, it is not known whether this is the only source of ascending thin limb cells or whether cell proliferation occurs in the segment undergoing transformation. This study was designed to address these questions and to identify sites of cell proliferation in the loop of Henle. Rat pups, 1, 3, 5, 7, and 14 d old, received a single injection of 5-bromo-2’-deoxy-uridine (BrdU) 18 h before preservation of kidneys for immunohistochemistry. Thick ascending and descending limbs were identified by labeling with antibodies against the serotonin receptor, 5-HT1A, and aquaporin-1, respectively. Proliferating cells were identified with an antibody against BrdU. BrdU-positive cells in descending and ascending limbs of the loop of Henle were counted and expressed as percentages of the total number of aquaporin-1–positive and 5-HT1A–positive cells in the different segments. In the developing kidney, numerous BrdU-positive nuclei were observed in the nephrogenic zone. Outside of this location, BrdU-positive tubule cells were most prevalent in medullary rays in the inner cortex and in the outer medulla. BrdU-labeled cells were rare in the papillary portion of the loop of Henle and were not observed in the lower half of the papilla after 3 d of age. BrdU-labeled nuclei were not observed in segments undergoing transformation or in newly formed ascending thin limb epithelium. It was concluded that the growth zone for the loop of Henle is located around the corticomedullary junction, and the ascending thin limb is mainly, if not exclusively, derived from cells of the thick ascending limb.

The mammalian kidney is composed of two embryologically distinct tissues: the nephrons, which are derived from the metanephric blastema, and the collecting duct system, which is derived from the ureteric bud (1,2). Anatomically, the nephron includes the glomerulus, the proximal and distal convoluted tubules, and the loop of Henle.

The loop of Henle is formed as an outgrowth from the proximal and distal tubule anlage of the nephron, close to the vascular pole of the glomerulus. Concomitant with the growth and development of the renal papilla, the loops of Henle grow and descend from the glomerulus toward the tip of the papilla (3–5). Although numerous mitoses have been reported in the anlage of the loop of Henle (4), the site or sites of cell proliferation during the growth and development of the loop have not been established.

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a process that starts just before the bend of the loop and proceeds toward the outer medulla (4,8).

A recent study from our laboratory demonstrated that the transformation of the ascending limb epithelium occurs by deletion of thick ascending limb cells by apoptosis, followed by a structural transformation of remaining thick ascending limb cells into the squamous epithelium of the ascending thin limb (8). However, it is not known whether this transformation is accompanied by cell proliferation in the newly formed epithelium. Neither is it known whether the thick ascending limb epithelium is the only source of ascending thin limb cells or whether cell proliferation occurs in the adjacent descending thin limb, followed by migration of cells into the ascending limb. Furthermore, our previous study does not rule out the presence of a putative as-yet-undetected stem cell that might also give rise to ascending thin limb cells.

The purpose of the present study was to identify the site(s) of cell proliferation in the loop of Henle and to establish whether there is evidence of cell proliferation in the epithelium undergoing transformation, in the newly formed ascending thin limb epithelium, or in the adjacent descending thin limb at the transition between the two epithelia.

Materials and Methods

Animals and Bromo-2'-Deoxy-Uridine Treatment

Sprague-Dawley rats were used in all experiments. Kidneys were obtained from 1-, 3-, 5-, 7-, and 14-d-old pups. For each age group, animals from two separate litters were used. All animals were administered 5-bromo-2'-deoxy-uridine (BrdU; Boehringer Mannheim GmbH, Mannheim, Germany), 50 μg/g body wt, as a single subcutaneous injection 18 h before preservation of kidneys for immunohistochemistry. BrdU is a thymidine analog that is incorporated into DNA during the S phase of the cell cycle and subsequently can be detected on tissue sections by use of specific antibodies against BrdU (9,10).

Tissue Preservation

The animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg body wt). The kidneys were preserved by in vivo perfusion through the heart. The animals initially were perfused briefly with phosphate-buffered saline (PBS), osmolality 298 mOsm/kg H2O (pH 7.4), to rinse away all blood. This was followed by perfusion with a periodate-lysine-parafomaldehyde solution for 5 min. After perfusion, the kidneys were removed and cut into 1- to 2-mm-thick slices that were fixed additionally by immersion in the periodate-lysine-parafomaldehyde solution overnight at 4°C. Sections of tissue were cut transversely through the entire kidney on a Vibratome Pelco 101, Sectioning series 1000 (Technical Products International, St. Louis, MO) at a thickness of 50 μm and processed for immunohistochemical studies by use of a horseradish peroxidase preembedding technique.

Antibodies

The thick ascending limb of the loop of Henle was identified by use of a rabbit polyclonal antibody against the human serotonin receptor, 5-HT1A (kindly provided by Dr. John Raymond, Medical University of South Carolina, Charleston, SC). The antibody labels the basolateral plasma membrane of all distal tubule cells, including those of the thick ascending limb, distal convoluted tubule, and connecting tubule (11). The proximal tubule and descending thin limb of the loop of Henle were identified by use of an affinity-purified rabbit polyclonal antibody raised against a synthetic peptide corresponding to the terminal 22 amino acids of rat aquaporin-1 (AQP1). The antibody recognizes AQP1 in the rat kidney and has been characterized in detail in previous studies (12). For the detection of BrdU, a mouse monoclonal antibody against BrdU (Boehringer Mannheim) was used.

Preembedding Immunolabeling for 5-HT1A Receptor

Fifty-μm Vibratome sections were processed for immunohistochemistry by use of an indirect preembedding immunoperoxidase method. All sections were washed with 50 mM NH4Cl in PBS, three times for 15 min. Before incubation with the primary antibody, the sections were pretreated with a graded series of ethanol for antigen retrieval and incubated for 2 h with PBS containing 1% bovine serum albumin (BSA), 0.05% saponin, and 0.2% gelatin (solution A). The tissue sections then were incubated overnight at 4°C with the antibody against the 5-HT1A receptor, diluted 1:50 in 1% BSA-PBS (solution B). 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, West Grove, PA), diluted 1:50 in solution B. The tissues then were rinsed, first in solution A and subsequently in 0.05 M tris(hydroxymethyl)aminomethane (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 H2O2 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, the sections were dehydrated in a graded series of ethanol and embedded in PolyBed 812 Resin (Polysciences Inc., Warrington, PA). From all animals, 50-μm-thick Vibratome sections through the entire kidney were mounted in Epon 812 between polyethylene vinyl sheets.

Postembedding Immunolabeling for AQP1 and BrdU

From flat-embedded 50-μm-thick Vibratome sections of kidney processed for immunohistochemical identification of the thick ascending limb of the loop of Henle, sections from the cortex, outer medulla, and outer and inner parts of the papilla were excised and glued onto empty blocks of Epon 812. Two consecutive 1-μm-thick sections were cut and treated for 10 to 15 min with a saturated solution of sodium hydroxide, diluted 1:3 in absolute ethanol, to remove the resin. After three brief rinses in absolute ethanol, the sections were hydrated with graded ethanol and rinsed in tap water. To increase accessibility of the DNA, the sections were treated with 20 μg/ml proteinase K (Fisher Biotech) containing 10 mM ethylenediaminetetraacetate and 10 mM NaCl in 0.05 M Tris buffer (pH 7.8) in a humidified chamber for 5 min at room temperature. This was followed by treatment with 0.2% glycine for 5 min to inhibit the enzyme. After being rinsed in tap water for 10 min, the tissue sections were incubated for 30 min with methanolic H2O2, rinsed in tap water for 10 min, and treated with 0.5% triton X-100 in PBS for 15 min. The sections then were rinsed in PBS three times for 10 min before being treated with 1% BSA for 1 h. One of the consecutive sections was incubated with the mouse anti-BrdU antibody (1:60), whereas the other section was incubated with a mixture of rabbit anti-AQP1 (1:500) and mouse anti-BrdU (1:60) antibodies overnight at 4°C. After being washed in PBS, the sections were incubated for 2 h in a mixture of peroxidase-conjugated goat anti-rabbit IgG and peroxidase-conjugated donkey anti-mouse IgG (Jackson ImmunoResearch Laboratories). For detection of peroxidase, Vector SG (Vector Laboratories, Burlingame, CA) was used as the chromogen to produce a gray-blue color, which is easily
distinguished from the brown staining produced by 3,3’-diaminobenzidine in the preembedding procedure used for detection of 5-HT_{1A}.

The sections were washed with distilled water, dehydrated with graded ethanol and xylene, mounted in Canada balsam, and examined by light microscopy.

**Quantitation of BrdU-Positive Cells**

Thick ascending limb cells, identified by basolateral 5-HT_{1A} receptor immunolabeling, and proximal tubule and descending thin limb cells, identified by AQP1 immunolabeling, were counted on 1-μm-thick semithin sections form the cortex, outer medulla, and proximal and distal papilla from four animals derived from two different litters at each of the following ages: 1, 3, 5, 7, and 14 d. On the same sections, 5-HT_{1A}–positive and AQP1-positive cells with BrdU-labeled nuclei were counted and expressed as percentages of the total number of 5-HT_{1A}– and AQP1-positive cells, respectively, counted in the different tubule segments for each animal. The values are presented as mean ± SD from four animals.

**Results**

**Identification of the Thick Ascending Limb**

Cells of the thick ascending limb were identified by labeling with antibodies to the serotonin receptor 5-HT_{1A}. In the developing kidney, 5-HT_{1A} immunoreactivity was observed in the basolateral plasma membrane of the distal nephron, including the thick ascending limb, distal convoluted tubule, and connecting tubule (Figures 1 through 4). At birth, 5-HT_{1A}–labeled thick ascending limbs were present throughout the renal medulla, including the papilla (Figures 3 and 4). There were no ascending thin limbs, and the squamous epithelium of the descending thin limb continued directly into the 5-HT_{1A}–positive cuboidal epithelium of the primitive thick ascending limb. From 1 to 14 d of age, the 5-HT_{1A}–positive cuboidal epithelium of the thick ascending limb in the renal papilla was transformed gradually into a 5-HT_{1A}–negative squamous epithelium with the structural characteristics of the ascending thin limb of the adult rat kidney, as demonstrated in a previous study from our laboratory (8). The transformation of the epithelium and disappearance of 5-HT_{1A} immunostaining started at the papillary tip and proceeded gradually in an ascending manner. It was accomplished by removal of numerous thick ascending limb cells by apoptosis and remodeling of the remaining epithelial cells (8).

**Identification of the Descending Limb of Henle’s Loop**

Descending limb cells were identified by immunolabeling with antibodies to AQP1. In developing nephrons, AQP1

![Figure 1](image-url). Differential interference contrast (DIC) micrographs of the outer cortex from 1- (A) and 14-d-old pups (B), illustrating double immunostaining for the 5-HT_{1A} receptor and 5-bromo-2′-deoxy-uridine (BrdU) in the distal tubule. BrdU-positive nuclei are present mainly in mesenchymal cells in the outer cortex of the 1-d-old pup (A). Only a few BrdU-labeled nuclei (arrows) were observed in the 5-HT_{1A}–positive distal tubule at both ages. Am, ampulla of ureteric bud. Magnification, ×660.
Figure 2. DIC micrographs of medullary rays in the renal cortex from 1- (A), 3- (B), 5- (C), and 14-d-old pups (D), illustrating double immunostaining for the 5-HT₁A receptor and BrdU in the cortical thick ascending limb (TAL). Numerous BrdU-positive cells (arrows) were present in the TAL during the first week of life (A through C), but labeled cells were rare in 14-d-old pups (D). Magnification, ×660.
immunoreactivity was confined to the plasma membrane of proximal tubules and the descending thin limb of the loop of Henle (Figures 5 through 8). At birth, the transition from the pars recta of the proximal tubule to the descending thin limb was subtle, with a gradual change in tubule diameter, a gradual decrease in cell height, and a loss of microvilli. From 3 to 7 d of age, there was an increase in the number of loops of Henle that all exhibited a gradual transition from the cuboidal epithelium of the proximal tubule to the squamous epithelium of the immature descending thin limb (Figure 6). At 14 d of age, there was an abrupt transition from the cuboidal epithelium of the proximal tubule to the squamous epithelium of the descending thin limb, thus defining the border between the outer and inner stripe of the outer medulla.

**Detection of Cell Proliferation**

Cell proliferation in the loop of Henle was determined by incorporation of BrdU into DNA of dividing cells followed by immunohistochemical detection by use of a double- and triple-labeling procedure for the detection of BrdU, 5-HT_{1A}, and AQP1 immunoreactivity on the same section. In all animals, cells with BrdU-positive nuclei, identified by their gray-blue color, were present throughout the renal cortex and outer medulla (Figures 1, 2, 3, 5, and 6). During the first week of life, numerous BrdU-labeled cells were located in the nephrogenic zone in the outer cortex (Figures 1A and 5). They were most prevalent in the undifferentiated mesenchyme but also were observed frequently in the ampullae and the ureteric bud as well as in developing glomeruli and tubules. Throughout kidney development, most of the BrdU-labeled tubule cells were located in medullary rays in the cortex (Figure 2) and in the outer medulla (Figures 3 and 6). BrdU-labeled cells were much less common in the renal papilla at any of the ages studied, and they were rarely observed in the terminal part of the papilla (Figures 4 and 8), where they seemed to represent mainly interstitial cells and collecting duct cells. In the 20 animals studied, a total of only two BrdU-positive cells were detected in the 5-HT_{1A}-positive epithelium of the thick ascending limb in the inner part of the renal papilla. There were no BrdU-positive nuclei in the 5-HT_{1A}-positive cells undergoing transformation in the ascending limb of Henle’s loop or in the already transformed epithelium that still exhibited weak

**Figure 3.** DIC micrographs of outer medulla from 1- (A), 5- (B), and 14-d-old pups (C), illustrating double immunostaining for the 5-HT_{1A} receptor and BrdU in the medullary TAL. Numerous BrdU-positive nuclei (arrows) were observed in the TAL at all ages, but the number was decreased at 14 d of age. Magnification, ×660.
5-HT_{1A} immunostaining. BrdU-labeled nuclei were rarely observed in the AQP1-positive descending thin limb in the renal papilla (Figures 7 and 8).

Quantitation of BrdU-Positive Nuclei in Ascending Limb of Henle’s Loop

The number of BrdU-positive nuclei, expressed as percentages of the total number of 5-HT_{1A}-positive cells in the ascending limb of the loop of Henle, are listed in Table 1. Among the 5-HT_{1A}-positive tubules, including the thick ascending limb, distal convoluted tubule, and connecting tubule, the largest number of BrdU-positive nuclei were observed in the cortical thick ascending limb in the medullary ray of the renal cortex at all ages studied (approximately 15% in 1-, 3-, and 5-d-old pups; 13% in 7-d-old pups; and 5% in 14-d-old pups). In contrast, there were few BrdU-labeled cells in 5-HT_{1A}-positive tubules in the cortical labyrinth (2 to 3% of the total number of 5-HT_{1A}-positive cells in the distal convoluted tubule and connecting tubule). Numerous cells with BrdU-labeled nuclei were observed in the 5-HT_{1A}-positive thick ascending limbs in the outer medulla of all animals (9 to 11% in 1- and 3-d-old pups and 6 to 7% in 5-, 7-, and 14-d-old pups). In sharp contrast, BrdU-labeled cells were rare (<3%) in 5-HT_{1A}-positive cells in the renal papilla at all ages, and they were absent in the lower half of the papilla after 3 d of age. BrdU-labeled nuclei were not observed in 5-HT_{1A}-positive cells undergoing apoptosis or in the 5-HT_{1A}-positive, newly formed ascending thin limb epithelium.

Quantitation of BrdU-Positive Nuclei in Descending Limb of Henle’s Loop

The number of BrdU-positive nuclei, expressed as percentage of the total number of AQP1-positive cells in the descending limb of the loop of Henle, are listed in Table 2. Among the AQP1-positive tubules, including the proximal tubule and descending thin limb, the largest number of BrdU-labeled nuclei was observed in the pars recta of the proximal tubule in the medullary ray (15 to 17% in 1- and 3-d-old pups and 7 to 8% in 5-, 7-, and 14-d-old pups) and outer medulla (13 to 20%) at all ages studied. In contrast, there were few BrdU-labeled cells in AQP1-positive proximal convoluted tubules in the cortical labyrinth (2 to 3%). BrdU-labeled cells were rare (<4%) in AQP1-positive immature descending thin limbs in both the outer medulla and the renal papilla at all ages, and they were absent from the lower half of the renal papilla after 3 d of age.

Discussion

The results of the present study demonstrate that the main sites of cell proliferation in both the descending and ascending
limbs of the loop of Henle in the developing rat kidney are located in the medullary ray in the cortex and in the outer stripe of the outer medulla. Cell proliferation was relatively rare in the papillary portion of the loop of Henle.

In a previous study of the developing rat kidney, we demonstrated that the ascending thin limb is derived from the epithelium of the primitive thick ascending limb by a process that involves apoptotic deletion of thick ascending limb cells in the inner medulla, followed by transformation of the remaining thick ascending limb cells into a thin squamous epithelium (8). However, these observations did not rule out the possibility that ascending thin limb cells might derive from proliferating cells in the adjacent descending thin limb or by proliferation of already transformed thick ascending limb cells or as-yet-identified stem cells in the segment undergoing transformation. The present study was designed to test these possibilities by identifying sites of cell proliferation in the loop of Henle and in particular by determining whether proliferating cells could be detected in or adjacent to the segment undergoing transformation. The results, which are summarized in Figure 9, demonstrate that cell proliferation in the loop of Henle occurs mainly in the outer medulla and in medullary rays in the cortex. In the renal papilla, <4% of the cells in the loop of Henle were undergoing proliferation, and, in the terminal part of the renal papilla, cell proliferation was not observed in the loop of Henle after 3 d of age. Cell proliferation was not observed in the transforming part of the primitive thick ascending limb that contained apoptotic cells or in the newly formed ascending thin limb epithelium, which was easily identified because 5-HT1A immunoreactivity was retained for a short period of time after the transformation occurred. These observations indicate that ascending thin limb cells are not derived from unidentified stem cells located in the immature thick ascending limb epithelium undergoing transformation. Moreover, the absence of detectable BrdU-labeled cells in the already transformed epithelium indicates that the ascending thin limb is not a major site of cell proliferation. Finally, the absence of detectable cell proliferation in the AQP1-positive descending thin limb immediately adjacent to the epithelium undergoing transformation indicates that ascending thin limb cells are not derived from proliferating cells in the descending limb of Henle’s loop. It is generally accepted that the cells of the descending limb of the loop of Henle are derived from the proximal tubule anlage of the S-shaped body, whereas the ascending limb is derived from the distal tubule anlage (3–5). Our results confirm that the boundary between the descending and ascending limb epithelium is located shortly before the bend of the loop in the terminal part of the descending limb, and there is no evidence that descending thin limb cells are being transformed into ascending thin limb cells. Taken together, these observations support the results of previous studies from our laboratory (8) and by other investigators (4,5) suggesting that the ascending thin limb is derived mainly by a transformation of the immature thick ascending limb epithelium in the renal papilla during the first 2 wk after birth.

The results of the present study also revealed that cell proliferation in both the descending and ascending limb of the
Figure 6. DIC micrographs of serial sections of OM from 1- (A and A'), 3- (B and B'), and 7-d-old pups (C and C'), illustrating double immunostaining for 5-HT<sub>1A</sub> and BrdU (A, B, and C) and triple immunostaining for 5-HT<sub>1A</sub>, AQP1, and BrdU (A', B', and C'). Numerous BrdU-positive cells (arrows) are present in both the 5-HT<sub>1A</sub>-positive TAL (∗) and the AQP1-positive thin descending limb (*). Magnifications: ×530 in A, A', C, and C'; ×500 in B and B'.
Figure 7. DIC micrographs of the outer part of the renal papilla from 1- (A and B), 7- (C), and 14-d-old pups (D), illustrating double immunostaining for 5-HT$_{1A}$ and BrdU (A) and triple immunostaining for 5-HT$_{1A}$, AQP1, and BrdU (B through D). A few BrdU-labeled cells (arrows) are present in the 5-HT$_{1A}$-positive TAL (★) in 1-d-old pups (A). However, BrdU-labeled cells were not observed in ascending limbs undergoing transformation (★ in C). BrdU-labeled cells were rare (none in this figure) in the AQP1-positive descending thin limb (*). Magnification, ×530.
Figure 8. DIC micrographs of the inner (terminal) part of the renal papilla from 1- (A and B), 5- (C), and 7-d-old pups (D), illustrating double immunostaining for 5-HT$_{1A}$ and BrdU (A) and triple immunostaining for 5-HT$_{1A}$, AQP1, and BrdU (B through D). BrdU-labeled cells were not observed in transforming TAL (★) or in AQP1-positive descending thin limb (*). Arrowhead indicates apoptotic bodies. Magnification, ×530.
The loop of Henle occurs mainly in the medullary ray of the renal cortex and in the outer stripe of the outer medulla. Thus, two distinct areas of cell proliferation exist in the developing kidney: the nephrogenic zone in the outer cortex under the renal capsule and the area around the corticomedullary junction, which appears to constitute a major growth zone for medullary structures, including the loop of Henle. In the developing kidney, the transition from the proximal tubule to the descending thin limb is initially subtle with a gradual decrease in cell height and loss of the brush border (4). Cell proliferation was detected in the part of the descending limb corresponding to the primitive or immature S3 segment of the proximal tubule. Of interest, in adult animals, proximal tubules and thick ascending limbs that are located in medullary rays in the cortex and in the outer stripe of the outer medulla are especially sensitive to ischemic as well as nephrotoxic injury (13). The recovery phase from acute renal failure in these conditions is characterized by significant cell proliferation in the pars recta of the proximal tubule as well as the thick ascending limb in this region (14–16). Thus, it appears that the capacity to undergo cell proliferation is preserved in these segments of the nephron throughout life. The low percentage of proliferating cells observed in the loop of Henle in the outer part of the renal papilla during the first three days after birth was very similar to that found in both proximal and distal convoluted tubules throughout postnatal development. After 3 d of age, cell proliferation was rare in the papillary portion of the loop of Henle. The rarity of proliferating cells in the renal papilla is in agreement with the results of previous studies reporting a very low mitotic count.

Table 1. BrdU-labeled cells in the 5-HT1A receptor-positive ascending limb of loop of Henle in the postnatal rat kidney

<table>
<thead>
<tr>
<th>Location</th>
<th>1 D</th>
<th>3 D</th>
<th>5 D</th>
<th>7 D</th>
<th>14 D</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL</td>
<td>3.3 ± 1.5</td>
<td>2.6 ± 1.5</td>
<td>3.1 ± 0.5</td>
<td>2.4 ± 1.0</td>
<td>1.6 ± 1.0</td>
</tr>
<tr>
<td>MR</td>
<td>14.5 ± 5.3</td>
<td>14.9 ± 3.6</td>
<td>14.6 ± 4.1</td>
<td>12.5 ± 4.6</td>
<td>5.0 ± 0.8</td>
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<tr>
<td>OM</td>
<td>9.0 ± 4.1</td>
<td>10.8 ± 2.2</td>
<td>7.2 ± 2.6</td>
<td>6.4 ± 2.5</td>
<td>6.4 ± 3.4</td>
</tr>
<tr>
<td>OP</td>
<td>3.2 ± 2.6</td>
<td>2.2 ± 1.5</td>
<td>1.5 ± 0.9</td>
<td>0.8 ± 1.5</td>
<td>0.4 ± 0.8</td>
</tr>
<tr>
<td>IP</td>
<td>0.1 ± 0.2</td>
<td>0.03 ± 0.08</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Values are mean ± SD and are expressed as a percentage of all 5-HT1A receptor-positive ascending limb cells. BrdU, 5-bromo-2’-deoxy-uridine; CL, cortical labyrinth (distal convoluted tubule and connecting tubule); MR, medullary ray; OM, outer medulla; OP, outer renal papilla; IP, inner renal papilla.

Table 2. BrdU-labeled cells in the AQP1-positive descending limb of loop of Henle in the postnatal rat kidney

<table>
<thead>
<tr>
<th>Location</th>
<th>1 D</th>
<th>3 D</th>
<th>5 D</th>
<th>7 D</th>
<th>14 D</th>
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</thead>
<tbody>
<tr>
<td>CL</td>
<td>2.8 ± 1.2</td>
<td>3.0 ± 2.1</td>
<td>3.2 ± 0.8</td>
<td>2.7 ± 1.4</td>
<td>1.5 ± 0.4</td>
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<tr>
<td>MR</td>
<td>17.1 ± 7.5</td>
<td>15.1 ± 3.5</td>
<td>7.2 ± 2.9</td>
<td>8.0 ± 1.5</td>
<td>6.8 ± 2.8</td>
</tr>
<tr>
<td>OM (OS)</td>
<td>12.5 ± 1.9</td>
<td>19.7 ± 3.0</td>
<td>15.2 ± 0.6</td>
<td>14.0 ± 5.4</td>
<td>12.9 ± 6.0</td>
</tr>
<tr>
<td>OM (IS)</td>
<td>3.1 ± 2.8</td>
<td>4.0 ± 3.5</td>
<td>2.7 ± 0.5</td>
<td>1.4 ± 0.5</td>
<td>0.8 ± 1.3</td>
</tr>
<tr>
<td>OP</td>
<td>3.4 ± 1.8</td>
<td>2.8 ± 3.0</td>
<td>0.8 ± 0.9</td>
<td>1.4 ± 0.6</td>
<td>0</td>
</tr>
<tr>
<td>IP</td>
<td>0.9 ± 1.5</td>
<td>0.6 ± 1.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Values are mean ± SD and are expressed as a percentage of all AQP1-positive descending limb cells. AQP1, aquaporin-1; OS, outer stripe; IS, inner stripe.

Figure 9. The development of the loop of Henle. The descending limb of Henle’s loop is shown in blue. The thick ascending limb of Henle’s loop is shown in yellow. The open arrow points to thick ascending limb cells undergoing apoptosis and transformation into ascending thin limb epithelium, which is shown in red. The intensity of purple in the tubules indicates the prevalence of BrdU-positive cells.
index in the papilla of the developing rat kidney (17,18). Studies in the developing rat kidney by Coles et al. (17) reported that, at all ages examined, mitotic figures were mainly observed in the nephrogenic zone in the renal cortex. However, results were presented only from the nephrogenic zone and from the renal papilla, where mitotic figures were very rare. There was no information about cell proliferation in the inner cortex or outer medulla. In contrast, Kahane et al. (18) reported that in 10-d-old rats, the highest mitotic index was found in tubules in the renal medulla, which did not include the papilla. This indicates that cell proliferation was observed in the outer medulla, which is in agreement with the results of our study. In a study of cell proliferation in the developing human kidney, Nadasdy et al. (19) reported that the highest proliferation index was observed in immature tubules in the nephrogenic zone and in the distal nephron, including the thick ascending limb and distal convoluted tubule. However, no distinction was made between the different regions of the kidney.

In summary, the main site of cell proliferation in both the descending and ascending limb of the loop of Henle was found in the medullary ray in the inner cortex and in the outer stripe of the outer medulla. There was no evidence for the presence of stem cells or proliferating cells in the segments undergoing transformation. We conclude that the growth zone for the loop of Henle is located at the corticomedullary junction, and the ascending thin limb is derived mainly, if not exclusively, from cells of the primitive thick ascending limb.

Acknowledgments

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