Expression of aldose reductase in developing rat kidney

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Expression of aldose reductase in developing rat kidney. Am J Physiol Renal Physiol 283: F481–F491, 2002. First published March 26, 2002; 10.1152/ajprenal.00332.2001. —Newborn rats are not capable of producing concentrated urine. With development of the concentrating system and a hypertonic medullary interstitium, intracellular osmolytes, such as sorbitol, accumulate in the renal medulla. Sorbitol is produced from glucose in a reaction catalyzed by aldose reductase (AR). The purpose of this study was to establish the time of expression and distribution of AR in the developing rat kidney. Kidneys from 16-, 18-, and 20-day-old fetuses and 1-, 3-, 4-, 5-, 7-, 14-, and 21-day-old pups were processed for immunohistochemistry and immunoblot analysis. In adult animals, AR was expressed only in the inner medulla, in which it was localized in ascending thin limbs (ATLs), inner medullary collecting ducts (IMCDs), and interstitial cells. AR immunoreactivity was not detected in fetal kidneys but was observed in the terminal part of the descending thin limb and IMCD in the renal papilla of 1-day-old pups. At birth, all of the loops of Henle are configured as short loops and there are no ATLs. After birth, papillary thick ascending limbs are gradually transformed into ATLs by a process that involves apoptotic deletion of cells from the thick ascending limb. During this time, AR immunoreactivity appeared in the cells undergoing transformation in the ascending limb, beginning at the papillary tip and ascending to the border between the outer medulla and the inner medulla. However, there was no labeling of apoptotic cells. The expression of AR in both the ATL and the IMCD gradually increased during kidney development. We conclude that AR expression in the inner medulla coincides with the increase in medullary tonicity that is known to occur during the first 3 wk after birth. On the basis of the observation that only AR-negative cells were deleted by apoptosis in the differentiating ATL, we propose that AR may protect ATL cells against apoptosis.

ascending thin limb; development; apoptosis

NEONATAL MAMMALS, including rats, are unable to concentrate their urine, but develop this ability after birth (4). Urinary osmolality in neonatal rats rises from 300 mosmol/kgH2O at birth to nearly 2,000 mosmol/kgH2O by 3 wk of age (23, 32). With the development of the urine-concentrating system and the associated increase in osmolality in the renal papilla (9, 30), the cells in this region need to generate intracellular compatible osmolytes, such as sorbitol, myo-inositol, betaine, taurine, and glycero-phosphorylcholine, for the maintenance and regulation of the intracellular milieu (2, 12, 31). Sorbitol, one of these osmolytes, is produced from D-glucose in an NADPH-dependent reduction reaction catalyzed by aldose reductase (AR).

It is well known that AR activity correlates with intracellular sorbitol concentration and inner medullary osmolality (25, 26). In the adult rat kidney, AR activity is mainly found in the inner medulla and increases toward the papillary tip. There is no evidence of AR activity in the renal cortex and outer medulla. In the neonatal kidney, AR mRNA expression as well as enzyme activity in the inner medulla is significantly lower than in the adult kidney (1). Schwartz et al. (27) have demonstrated that AR mRNA and AR activity in terminal inner medullary collecting ducts (IMCDs) microdissected from developing rats increased dramatically during the first and second weeks after birth.

An increase in the ability to concentrate urine in the neonatal kidney is closely related to the morphological maturation of the renal papilla, especially the loop of Henle (9). In the neonatal kidney, the renal medulla is not separated into outer and inner zones. At the time of birth, ascending limbs with immature distal tubule epithelium are present throughout the renal medulla. All of the loops of Henle have the structural characteristics of the short loops of the adult kidney, and there are no ascending thin limbs (ATLs). Our laboratory (14) has previously demonstrated that immature thick ascending limbs (TALs) in the renal papilla are transformed into ATLs after apoptotic deletion of cells and differentiation of the remaining cells into a thin squamous epithelium. Thus the maturation of the loop of Henle and the development of a true inner medulla occur at the time of development of a hypertonic medullary interstitium.

In adult animals, it is well established that AR is expressed not only in the IMCD but also in the loop of...
Henle in the inner medulla (25, 29). However, little is known about the expression and distribution of AR in the developing kidney, and there is no information about AR in the differentiating loop of Henle. Therefore, our study was designed to establish the time of expression and the pattern of distribution of AR in the developing rat kidney, with special focus on the developing loop of Henle.

### METHODS

#### Animals and Tissue Preservation

Sprague-Dawley rats were used in all experiments. The kidneys were obtained from 16-, 18-, and 20-day-old fetuses (E16, E18, and E20, respectively) and 1-, 3-, 4-, 5-, 7-, 14-, and 21-day-old pups (P1, P3, P4, P5, P7, P14, and P21, respectively). For each age group, three or four animals derived from two separate litters were used. The animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg). The kidneys were preserved by in vivo perfusion through the heart or abdominal aorta. The animals were initially perfused briefly with PBS (298 mosmol/kgH2O, pH 7.4) to rinse away blood. This was followed by perfusion with a periodate-lysine-2% paraformaldehyde 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 periodate-lysine-2% paraformaldehyde solution overnight at 4°C. Sections of tissue were cut transversely.

### Table 1. Double labeling by immunohistochemistry

<table>
<thead>
<tr>
<th>Protein Labeled</th>
<th>Section I</th>
<th>Section II</th>
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<tr>
<td>5-HT1A</td>
<td>DAB</td>
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<tr>
<td>AR</td>
<td>Vector SG</td>
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<td>AQP1</td>
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5-HT1A, 5-hydroxytryptamine 1A; AR, aldose reductase; AQP1, aquaporin-1; DAB, 3,3'-diaminobenzidine.
through the entire kidney on a vibratome at a thickness of 50 μm and processed for immunohistochemical studies using a horseradish peroxidase preembedding technique.

**Antibodies**

AR immunoreactivity was detected using an affinity-purified goat polyclonal antibody against AR from rat lens (courtesy of Dr. Peter Kador, National Institutes of Health, Bethesda, MD). The antibody has been characterized in a previous study (29). The TAL was identified using a rabbit polyclonal antibody against the human serotonin receptor 5-hydroxytryptamine 1A (5-HT1A; courtesy of Dr. John Raymond, Duke University, Durham, NC). This antibody labels the basolateral plasma membrane of the TAL, distal convoluted tubule, and connecting tubule cells (24). The descending thin limb (DTL) of the loop of Henle was identified using a rabbit polyclonal antibody to aquaporin-1 (AQP1; courtesy of Dr. Mark A. Knepper, National Institutes of Health). This antibody labels the apical and basolateral plasma membrane of the proximal tubule and DTL (22).

**Immunohistochemistry**

Fifty-micrometer vibratome sections were processed for immunohistochemistry using an indirect preembedding immunoperoxidase method. All sections were washed with 50 mM NH₄Cl in PBS three times for a total of 15 min. Before incubation with the primary antibody, the sections were pretreated with PBS containing 1% BSA, 0.05% saponin, and 0.2% gelatin (solution A) for 3 h. They were then incubated overnight at 4°C with antibodies to AR (1:100,000) or 5-HT1A (1:1,000) diluted in 1% BSA in PBS (solution B). The tissue labeled with antibodies against 5-HT1A is the same as that used in a previous study of the development of the ATL (14). Control incubations were performed in solution B lacking primary antibody. After three washes with solution A, the sections were incubated for 2 h in horseradish peroxidase-conjugated donkey anti-goat or anti-rabbit IgG Fab fragment (Jackson ImmunoResearch Laboratories, West Grove, PA) diluted 1:100 in solution B. The tissues were rinsed first in solution A and then in 0.05 M Tris buffer (pH 7.6). For the detection of horseradish peroxidase, 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 being washed with 0.05 M Tris buffer, the sections were dehydrated in a graded series of ethanol. From all animals, 50-μm-thick vibratome sections through the entire kidney were used.

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**Fig. 2.** Light micrographs of 50-μm-thick vibratome sections illustrating immunostaining for AR in renal papilla of kidneys from a 20-day-old fetus (A) and 1-, 7-, 14-, and 21-day-old pups (B–F, respectively). A: in 20-day-old fetal kidneys, there was no immunoreactivity for AR. B: at birth, AR immunoreactivity first appeared in the terminal part of the descending thin limb (DTL) and the terminal part of the medullary collecting duct. C: higher magnification demonstrated that the AR-positive DTL (*) continued directly into the TAL (†). Note the absence of ATL in all of the loops of Henle at this stage. D–F: AR-positive tubules gradually ascended to the future border (dashed lines) between the outer medulla and the inner medulla. Magnification: A, B, and D–F, ×24; C, ×240.
were embedded in Poly/Bed 812 resin (Polysciences, War-
rington, CA) sandwiched between polyethylene vinyl
sheets.

**Double Labeling**

From the flat-embedded vibratome sections of kidneys
processed for immunohistochemical identification of TAL us-
ing 5-HT\textsubscript{1A}, different areas of the renal medulla were excised
and glued onto an empty block of Poly/Bed 812 resin. Three
consecutive 1.5-\mu m sections were cut for double immuno-
labeling for AR or AQP1 using a postembedding technique. The
sections were treated for 5 min with a mixture of saturated
sodium hydroxide and absolute ethanol (1:1) to remove the
resin. After three brief rinses in absolute ethanol, the sec-
tions were hydrated with graded ethanol and rinsed in tap
water. The sections were then rinsed with PBS, incubated in
normal donkey serum (Jackson ImmunoResearch Laborato-
ries) for 1 h, and subsequently incubated overnight at 4°C
with antibodies to either AR or AQP1. After being washed in
PBS, the sections were incubated for 2 h in peroxidase-
conjugated donkey anti-goat or anti-rabbit IgG (Fab frag-
ment) and washed again with PBS. For detection of AR and
AQP1, Vector SG (Vector Laboratories, Burlingame, CA) was
used as the chromogen to produce a gray-blue color, which is
easily distinguished from the brown label produced by 3,3'-
diaminobenzidine in the first immunolocalization procedure
for 5-HT\textsubscript{1A} using the preembedding method (Table 1). The
sections were washed with distilled water, dehydrated with
graded ethanol and xylene, mounted in balsam, and exam-
ined by light microscopy.

Fig. 3. Confocal microscopy using single
immunofluorescence labeling of AR
(A–E', F', and H'), 5-hydroxy-
tryptamine 1A (5-HT\textsubscript{1A}, E" and F"')
and aquaporin-1 (AQP1; G" and H"'),
and double immunofluorescence label-
ing of AR and 5-HT\textsubscript{1A} (E''' and F'''') or
AR and AQP1 (G'''' and H''''') in the
renal papilla of kidneys from 20-day-
old fetuses (A) and 1B, E, and G'-, 3
(D'), and 5-day-old (F and H') pups. A:
in 20-day-old fetal kidneys, there was
no immunoreactivity for AR. B: at
birth, AR immunoreactivity first ap-
peared strongly in the terminal part of
the DTL (*) and weakly in the terminal
part of the IMCD. C and D: note a
gradual increase in AR immuno-
reactivity in IMCD cells during develop-
ment. E'–E'': terminal part of renal
papilla of 1-day-old pup. Strong AR
(green fluorescence)-positive DTLs (*)
continue directly into 5-HT\textsubscript{1A} (red flu-
earence)-positive TALs (\(\times\)) in the
middle part of renal papilla of 5-day-
old pup. Note strong AR immunoreac-
tivity in the transformed ATL (\(\times\)) with
5-HT\textsubscript{1A} immunoreactivity (arrows).
G'–G''': at birth, AQP1 (red flu-
earence)-positive DTLs (*) also have AR
(green immunofluorescence) immuno-
reactivity. H'–H''': at 5 days after
birth, AR immunoreactivity is not seen
in the AQP1-positive DTLs (*) but was
observed in the AQP1-negative ATLs
(\(\times\)). Bar = 50 \mu m.
Confocal Laser Scanning Microscopy

For immunofluorescence microscopy, kidney blocks containing all kidney zones were dehydrated and embedded in wax (polyethylene glycol 400 disterate, Polysciences). The wax-embedded tissues were cut to 5 μm on a rotary microtome (Leica), and the sections were dewaxed and rehydrated. In double-label fluorescent studies, AR was localized with goat polyclonal antibodies, which were mixed with rabbit antibodies against 5-HT1A or AQP1, respectively. The labeling was visualized using a fluorescein (FITC)-conjugated donkey-anti goat antibody (diluted 1:50; Jackson ImmunoResearch Laboratories) mixed with a Cy3-conjugated donkey anti-rabbit antibody (diluted 1:500; Jackson ImmunoResearch Laboratories). The microscopy was carried out using an MRC-1024 laser confocal microscope (Bio-Rad).

Toluidine Blue Staining

Apoptotic cells were identified on 1.5-μm plastic sections of tissue processed for immunohistochemical identification of the TAL and DTL of Henle’s loop. The sections were stained with toluidine blue, a vital dye known to stain cells undergoing apoptosis, and subsequently etched by incubation in a saturated solution of sodium hydroxide for 5 min, as described previously (14). After three brief rinses in absolute ethanol and a rinse with xylene, the sections were mounted in balsam and examined by light microscopy.

Western Blot Analysis

The renal cortex and medulla from three animals in each age group were homogenized in lysis buffer containing 20 mM Tris-HCl, 1% Triton X-100, 150 mM sodium chloride, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 0.02% sodium azide, 1 mM EDTA, 10 μM leupeptin, and 1 mM phenylmethylsulfonyl fluoride. The homogenate was centrifuged at 3,000 g for 20 min at 4°C. After determination of protein concentration in the supernatant by the Coomassie method (Pierce, Rockford, IL), samples were loaded (30 μg/ lane) and underwent electrophoresis on sodium dodecyl sul-
fate-polyacrylamide gels under reducing conditions. Proteins were transferred by electroelution to nitrocellulose membranes that had been blocked with 5% nonfat dry milk in PBS-T (0.1% Tween 20 in 0.01 M PBS, pH 7.4) for 30 min at room temperature, and the membranes were then incubated for 24 h at 4°C with affinity-purified anti-AR antibodies (1:100,000). The membranes were washed in several changes of PBS-T and incubated for 1 h with horseradish peroxidase-conjugated donkey anti-goat IgG (1:1,000). After a final washing, antibody labeling was visualized using an enhanced chemiluminescence system (Amersham Life Sciences, Buckinghamshire, UK) at room temperature.

RESULTS

Expression of AR in Adult Rat Kidney

AR immunoreactivity was present in the inner medulla, and there was no labeling in the cortex or outer medulla (Fig. 1A). Strong AR immunoreactivity was observed in the ATL, and there was an abrupt transition from the intensely labeled ATL to the AR-negative TAL, marking the border between the outer medulla and the inner medulla (Fig. 1, B and C). In the ATL, the intensity of AR immunoreactivity gradually decreased toward the tip of the papilla. AR was also expressed in the lower half of the IMCD and in interstitial cells in the deep papilla (Fig. 1D).

Expression of AR in Developing Rat Kidneys

Fetal kidney. At E16, E18, and E20, no AR immunoreactivity was detected in the cortex, outer medulla, or inner medulla (Figs. 2A and 3A).

Neonatal rat kidney. AR-positive tubular profiles appeared first in the terminal part of the renal papilla at P1 and ascended gradually to the future border be-

Fig. 5. Light micrographs of consecutive 1.5-μm sections from the outer (A and B), middle (C and D), and inner parts (E and F) of the renal papilla from a 4-day-old pup, illustrating double immunostaining for AQP1 and 5-HT1A (A, C, and E) and AR and 5-HT1A (B, D, and F). AR immunoreactivity is present in the 5-HT1A-positive TAL epithelium (**) undergoing differentiation from cuboidal to flat squamous epithelium in the middle and inner part of the renal papilla. AR immunoreactivity was not observed in the AQP1-positive DTL (*). Note that AR immunoreactivity increased slightly in the IMCDt. Magnification, ×528.
between the outer medulla and the inner medulla by 3 wk after birth (Figs. 2, B–F, and 3B).

By P1, all the loops of Henle had the configuration of short loops, and there were no ATLs (Fig. 2C). In the papillary tip, AR immunoreactivity was intense in the terminal part of the DTL (Fig. 3B), which was directly connected with the TAL, and weaker labeling was observed in the terminal part of the medullary collecting duct (Fig. 3B). To establish the exact sites of immunostaining for AR in the developing loop of Henle, a double-immunolabeling procedure was used in which the TAL and DTL were identified with antibodies to 5-HT1A and AQP1, respectively. In the base of the renal papilla, there was no AR immunoreactivity in the AQP1-positive DTL (Fig. 4, A and B). In contrast, there was little or no AQP1 immunoreactivity in the terminal part of the DTL in the papillary tip, in which AR immunolabeling was intense (Fig. 4, C and D). There was no AR immunoreactivity in 5-HT1A-positive TAL in the renal papilla at this age (Fig. 4, A–D).

In the kidneys from 3- to 7-day-old pups, AR immunoreactivity in the loop of Henle was mainly located in the ascending limb, which was in the process of differentiating from 5-HT1A-positive cuboidal epithelium to 5-HT1A-negative squamous epithelium (Figs. 3F, 5, and 6). By contrast, AR immunoreactivity in the terminal part of the DTL gradually disappeared and was not seen after 4 days of age (Figs. 3H and 5, C–F). In the transforming primitive TAL, AR-positive cells first appeared at the papillary tip and gradually increased in number in an ascending manner (Figs. 5 and 6). There was a gradual increase in AR immunoreactivity in both IMCD cells and interstitial cells in the terminal part of the papilla during development (Figs. 3, C–D, and 5).

In the kidneys of 14- and 21-day-old pups, most of the 5-HT1A-positive TAL epithelium in the renal papilla had been transformed into a 5-HT1A-negative squamous epithelium. There was strong AR immunoreactivity in the newly formed ATL cells (Fig. 6). The expression pattern of AR immunoreactivity in both IMCD cells and interstitial cells was similar to that seen in adult kidneys.

**Relationship of Expression of AR and Apoptosis in the Transforming Ascending Limb of the Loop of Henle**

For identification of apoptosis by light microscopy, plastic sections were stained with toluidine blue followed by etching with a sodium hydroxide solution. To establish the exact sites of apoptosis in the transforming ascending limb of the loop of Henle, the TAL was identified by immunolabeling with an antibody to 5-HT1A using a preembedding method. This was followed by labeling on two consecutive 1.5-μm sections of the same tissue using a postembedding method. The first section was stained with toluidine blue for detection of apoptotic cells and apoptotic bodies, and the
second section was used for detection of AR immunoactivity.

There were numerous apoptotic cells and apoptotic bodies in the 5-HT1A-positive TAL undergoing transformation in the renal papilla 2 wk after birth (Fig. 7). AR was not expressed in apoptotic cells or apoptotic bodies, but there was weak AR immunostaining in the remaining cells, which were differentiating into the strongly AR-positive ATL epithelium (Fig. 7).

**Western Blot Analysis**

Determination of AR expression by immunoblotting revealed a band at 36.5 kDa (Fig. 8). AR protein was expressed in the renal medulla of both neonatal and adult rats. However, AR was not detected in either cortex or outer medulla from animals at any age examined. AR was not expressed in fetal kidneys, but a faint band was observed in protein from the renal medulla at postnatal day 1. Determination of the relative abundance of AR protein by densitometry demonstrated a gradual increase in AR expression during the first 3 wk after birth.

**DISCUSSION**

The purpose of the present study was to determine the exact sites of AR expression in the developing kidney, especially in the differentiating loop of Henle, using specific markers to identify the cells of the DTL and TAL. At all ages of animals examined, AR was expressed only in the inner medulla, in which it was detected in both ATL and IMCD. AR immunoreactivity was first observed in the terminal part of the renal papilla in 1-day-old pups, and the intensity of labeling gradually increased during the first 3 wk after birth. From 1 to 14 days of age, AR immunolabeling appeared in the transforming ascending limb, beginning at the
papillary tip and ascending to the border between the outer medulla and the inner medulla (Fig. 9). In the transforming ascending limb epithelium, AR was not observed in those cells undergoing apoptosis but was expressed weakly in the remaining cells, which were differentiating into the strongly AR-positive ATL epithelium. These findings suggest that AR may play a role in the differentiation of the ATL. Moreover, it is

Fig. 8. Western blot demonstrating AR protein expression in the renal medulla (Me) of the developing rat kidney. Protein (30 μg) was applied to each lane. The antibody against AR identified a band at 36.5 kDa. There was a gradual increase in AR expression from neonatal to adult (Ad) kidney. Co, Cortex; F20, 20-day-old fetus; P1, P4, P7, P14, P21, 1-, 4-, 7-, 14-, and 21-day-old pups, respectively.

Fig. 9. Diagram illustrating expression of AR in a differentiating loop of Henle during development of the rat kidney. Arrows, apoptosis. Red, AR; blue, AQP1; yellow, 5-HT1A.
noteworthy that the expression of AR in the ATL and IMCD occurs during the first 2–3 wk after birth, when medullary tonicity is known to increase (23, 30).

The ability of the rat neonate to concentrate urine to adult levels is not reached until 3 wk after birth. There are a number of anatomical factors that contribute to the impaired concentrating ability of the neonatal kidney. Compared with adult, the neonate has poorly developed medullary vasa recta, abundant interstitial material in the medulla, and a shorter papilla in which there is no true inner medulla (30). The maturation of the concentrating system in the rat during the first 3 wk of postnatal life is associated with a lengthening of the renal papilla, including the long loops of Henle and the IMCD, and an increase in papillary sodium and urea concentrations (23, 32). Intracellular osmolytes, including sorbitol, are important for the maintenance of cellular functions in the hypertonic medullary interstitium, and sorbitol, which is generated from glucose by a process catalyzed by AR, is a major osmolyte in the inner medulla. In the neonatal rat, however, AR mRNA and activity in the inner medulla are significantly lower than in adult animals (1, 27).

It is well established that AR and various osmolyte transporters are induced by hyperosmotic stress (3, 12, 17, 31). Cells in the renal medulla are constantly exposed to steep osmotic gradients because of the high tonicity in the medullary interstitium required for the function of the urine-concentrating mechanism. Studies in cell lines derived from the inner medulla have demonstrated that increases in the osmolality of the medium are associated with increases in cellular sorbitol content, AR activity, and AR gene expression (6, 17, 21, 28). The mechanism for osmotic induction of AR gene transcription is not completely understood. Recently, however, Ferraris et al. (10), Ko et al. (16), and Daoudal et al. (7) identified a regulatory sequence element, the tonicity-responsive enhancer, in the AR gene of several species. Subsequently, Miyakawa et al. (20) characterized a tonicity-responsive enhancer binding protein, a transcription factor that regulates the expression of proteins catalyzing cellular accumulation of compatible osmolytes.

The role of osmotic stress in the induction of AR in the developing kidney is not known with certainty. Schwartz et al. (27) measured the changes in AR mRNA and activity in terminal IMCD microdissected from developing rat kidneys. These investigators found that AR mRNA and activity increased before a detectable increase occurred in urinary and inner medullary osmolality. On the basis of these observations, the authors concluded that the maturational induction of the AR gene was not a consequence of osmotic stimulation, but rather was part of the genetic program for the development of the kidney. However, it cannot be ruled out that small increases in inner medullary tonicity might have occurred that could not be detected. In this regard, it is noteworthy that a urea transporter is already expressed in the terminal IMCD 1 d after birth, suggesting that urea can be absorbed into the medullary interstitium in the distal part of the papilla shortly after birth (15).

Previous studies in adult animals have demonstrated AR immunoreactivity in Henle’s loop and the collecting duct of the inner medulla, whereas no immunoreactivity was observed in the cortex (18, 29). These results are in general agreement with the results of our study. However, the distribution pattern of AR immunoreactivity in the ATL is different from that in the IMCD. In the adult rat kidney, the intensity of AR immunoreactivity in the ATL is very strong in the initial part of the inner medulla but gradually decreases toward the tip of the renal papilla. In contrast, there is strong AR immunoreactivity in the IMCD cells and interstitial cells in the terminal part of the inner medulla but little or no labeling in the initial part of the inner medulla. These results suggest that the expression of AR in the inner medulla may be dependent on other factors in addition to hyperosmolality, at least in the ATL, or may be differently regulated in different cell types in the renal medulla.

In neonatal rat kidneys, immunostaining for AR was also observed in the transforming ascending limb, beginning at the papilla tip and ascending to the border between the outer medulla and the inner medulla. At birth, all of the loops of Henle in the rat renal papilla are configured as short loops and there is no ATL. During the first 2–3 wk after birth, the ATL is developed from the 5-HT1A-positive epithelium of the primitive TAL by a process that involves apoptotic deletion of AR-negative cells from the TAL and differentiation of the remaining tubule cells into the flat 5-HT1A-negative, but AR-positive, epithelium of the ATL (14). On the basis of the observation that only AR-negative cells in the papillary ascending limb are deleted by apoptosis, it is tempting to speculate that AR expression in the remaining ascending limb cells may play a role in protecting these cells from undergoing apoptosis.

It is not known what initiates apoptosis in the TAL at the tip of the renal papilla shortly after birth and causes the process to proceed in an ascending manner through the renal medulla during the first 3 wk of age. The demonstration that apoptosis occurs in distinct regions of the renal medulla at different points in time suggests that the process is activated by localized changes in environmental factors that may occur around the time of birth. There is increasing evidence that hypertonic stress induces apoptosis. Michea et al. (19) and Dmitrieva et al. (8) demonstrated that cell-cycle delay and apoptosis were induced by high NaCl and/or urea in murine IMCD cells. Thus it is possible that the increase in medullary tonicity that occurs after birth plays a role in the activation of the apoptotic process.

The results of this study support the idea that AR plays an important role in the ability of renal medullary cells to adapt to osmotic stress during renal development in neonatal rats. It remains to be established whether high intracellular ionic strength has direct effects on AR gene transcription in the renal medulla.
during kidney development. Our studies of the relationship between AR expression and apoptosis in the ascending limb of Henle’s loop suggest that AR may play a role in the differentiation of the ATL in the neonatal kidney. However, a direct relationship between AR and cell survival in the ascending limb remains to be established.

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