Collectrin, a Collecting Duct-specific Transmembrane Glycoprotein, Is a Novel Homolog of ACE2 and Is Developmentally Regulated in Embryonic Kidneys*

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Collectrin, a novel homolog of angiotensin-converting enzyme-related carboxypeptidase (ACE2), was identified during polymerase chain reaction-based cDNA subtraction and up-regulated in 5/6 ablated kidneys at hypertrophic phase. Collectrin, with 222 amino acids, has an apparent signal peptide and a transmembrane domain; the sequence is conserved in mouse, rat, and human and shares 81.9% identity. Human collectrin has 47.8% identity with non-catalytic extracellular, transmembrane, and cytosolic domains of ACE2; however, unlike ACE and ACE2, collectrin lacks active dipeptidyl carboxypeptidase catalytic domains. The collectrin mRNA transcripts are expressed exclusively in the kidney. In situ hybridization reveals its mRNA expression in renal collecting ducts, and immunohistochemistry shows that it is localized to the luminal surface and cytoplasm of collecting ducts. Immunoprecipitation studies, using [35S]methionine-labeled renal cortical cytoplasm, shows that it is localized to the luminal surface and cytoplasm of collecting ducts. Immunoprecipitation studies, using [35S]methionine-labeled renal cortical and inner medullar collecting duct cells, i.e., M-1 and mIMCD-3, indicate that the protein size is ~32 kDa. During the development of mouse kidney, mRNA signal is detectable at day 13 of gestation, and the protein product is observed in the ureteric bud branches. Its expression is progressively increased during later stages of the gestation extending into the neonatal periods and then is decreased in adult life. Up-regulated expression of collectrin in the hypertrophic kidneys after renal ablation and restricted spatio-temporal expression during development indicates a possible role(s) in the process of progressive renal failure and renal organogenesis.

Reduction of nephron number in various immunological and non-immunological renal diseases has been implicated in the development of subsequent glomerulosclerosis and interstitial scarring of the kidney that is followed by a decline in renal function. Such a reduction can be experimentally achieved by 5/6 renal ablations in rat, a model in which glomerular hyperfiltration in renal nephrons plays a central role in the development of glomerulosclerosis (1). In Wistar-Kyoto and Harlan Sprague Dawley rats, partial ablation of renal mass initiates a cycle of progressive renal injury in the remnant kidney associated with glomerular and tubular hypertrophy, hyperfiltration, and systemic hypertension (2). The remaining viable renal mass undergoes the following phases: 1) hypertrophic phase that occurs after 2–4 weeks of ablation, 2) the quiescent phase that follows during the next 4–10 weeks with minimal histological alterations, and 3) the development of segmental glomerular sclerosis and tubulointerstitial fibrosis that ensue after 10 weeks (3). To investigate the molecular mechanism(s) and the genes that may be involved in the initiation of such a pathobiologic response, we employed PCR-based subtractive hybridization method, i.e., representational difference analysis of cDNA (cDNA-RDA; Refs. 4 and 5) to screen the differentially expressed genes in remnant kidney at hypertrophic phase of 5/6 nephrectomized mice (6). In the process of cDNA subtraction, the cDNA fragment of a novel gene, NX-17, was isolated, and its mRNA expression was up-regulated in 5/6 ablated remnant kidney compared with control kidney (6).

In the present study, we have reported cDNA cloning of the full coding sequence of NX-17. The gene product of NX-17 has been designated as collectrin, because it is a novel transmembrane glycoprotein specifically expressed in collecting tubules of the kidney. With homology searches, collectrin was determined to be a novel homolog of ACE-related carboxypeptidase (ACE2; Refs. 7, 8), which has been recently identified as the human homolog of ACE (8). ACE2, like ACE, is a membrane-associated and secreted enzyme expressed predominantly on heart, kidney, and testis (8). ACE2 has a single carboxypeptidase active site and catalyzes the cleavage of angiotensin I (Ang I) to Ang1–9, whereas ACE has two active site domains and converts Ang I to angiotensin II (Ang II) (8). Collectrin shares 47.8% identity with C-terminal regions including non-catalytic extracellular, transmembrane, and cytosolic domains

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1 The abbreviations used are: PCR, polymerase chain reaction; ACE, angiotensin-converting enzyme; cDNA-RDA, representational difference analysis of cDNA; RACE, rapid amplification of 5′- and 3′-cDNA ends; Ang II, angiotensin II; AT1, angiotensin II type 1 receptor; Ras, renin-angiotensin system; ELISA, enzyme-linked immunosorbent assay; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; bp, base pair(s).
of ACE2, and this segment does not share homology with ACE. Although collectrin lacks catalytic domains, its highly restricted expression in collecting ducts and differential expression in embryonic and ablated kidneys suggest that it has a unique role in the pathobiology of collecting ducts that may be related to the organogenesis and organ failure of the kidney.

**EXPERIMENTAL PROCEDURES**

**Isolation of a Full-length Collectrin cDNA and Nucleotide Sequencing**—Using cDNA-RDA, we previously described the isolation of differentially expressed genes in the 5/6 nephrectomized mouse remnant kidney (3, 4). Among the various novel genes, NX-17 (collectrin partial-length cDNA) had a 2-fold up-regulated mRNA expression in nephrectomized mouse with 5/6 ablated kidney (5). Using this partial-length cDNA, the full coding sequence of collectrin cDNA from mouse, rat, and human was isolated with the Marathon cDNA amplification kit (CLONTECH, Palo Alto, CA). Total kidney RNAs from 8-week-old CD-1 (ICR) mice and 4-week-old Harlan Sprague Dawley rats (Charles River Co., Yokohama, Japan) were extracted by guanidinium isothiocyanate-CsCl ultracentrifugation (8–11), and mRNAs were further isolated by FastTrack 2.0 Kit (Invitrogen, San Diego, CA). Human kidney poly(A)1 RNA was purchased from CLONTECH. Double-stranded cDNAs were synthesized from 1.0 μg of mRNA and subjected to the rapid amplification of 5′- and 3′-untranslated regions (RACE) reactions using 5′-GCCCGCTGGAT-TATGTGGATCCTGGT-G3′ (506–533 bp, S1) and 5′-CATGTCCAAGG-GATCACAAGGGATGCC-3′ (669–695, AS1) as primers. The 5′- and 3′-RACE products were subcloned into the pCR2.1-TOPO vector (Invitrogen) and sequenced by automated DNA sequencing (ABI PRISM 310 Genetic Analyzer, Perkin-Elmer, Foster City, CA). At least four different clones were sequenced to ensure the fidelity of Taq polymerase.

**Structural and Homology Analysis**—Kyte and Doolittle hydrophobicity/hydrophilicity plot analysis of mouse collectrin. Two hydrophobic domains, i.e. signal peptide and transmembrane domain (TM), are noted. N-linked and O-linked glycosylation sites are indicated by N-Gly and O-Gly, respectively.
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KGPPOV, the sequence of which was derived from the amino acid stretch between residues 165 and 179 of the intracellular domain of collectrin (Fig. L A). This segment of collectrin is highly conserved and identical between the mouse and rat. The cysteine residue was added to the N terminus for conjugation of the peptide to keyhole limpet hemocyanin (KURAO, Osaka, Japan) to assess the specificity of the anti-collectrin antibody. ELISA (enzyme-linked immunosorbent assay) and competitive inhibition ELISA assays were performed as described previously (13).

Immunoprecipitation—SV40 MES 13 (glomerular mesangial cells from an SV40 transgenic mouse, CRL-1927, ATCC), and M-1 (kidney cortical collecting duct cells from an SV40 transgenic mouse, CRL-20336, ATCC) and mIMCD-3 (kidney inner medullar collecting duct cells, CRL-2123, ATCC) were grown in a mixture of Dulbecco’s modified Eagle’s medium, Ham’s F12 medium, and 5% fetal bovine serum. For immunoprecipitation, the cells were grown to ~70% confluence in a culture flask (~5 × 10^6 cells) and labeled with [35S]methionine and [35S]cysteine (0.25 mCi/ml, Amersham Pharmacia Biotech) for 12 h. The cells were washed with culture medium and lysed in immunoprecipitation buffer (IP buffer; 20 mM Tris-HCl, pH 7.4, 100 mM NaCl, 1 mM CaCl2, 1 mM MgCl2, 10 mM benzamidine-HCl, 10 mM e-amino-n-caproic acid, 2 mM phenylmethylsulfonyl fluoride, and 1% Triton X-100) by vigorous shaking at 4 °C for 2 h. The lysates were centrifuged at 12,000 × g for 30 min at 4 °C, and the supernatants were incubated with preimmune rabbit serum and protein A-Sepharose CL-4B (Amersham Pharmacia Biotech). For embryonic, newborn, and adult mouse kidneys, 20 µg of total RNA were used. Human 12-Lane MTN blot was purchased from CLONTECH. On this membrane, 2 µg of poly(A)+ RNA from human brain, heart, skeletal muscle, colon, thymus, spleen, kidney, liver, small intestine, placenta, lung, and peripheral blood leukocytes were blotted. The membranes were hybridized with [α-32P]dCTP-radiolabeled mouse, rat, and human collectrin, and GAPDH (1 × 10^6 cpm/ml) cDNA at 42 °C in ExpressHyb® Hybridization Solution (CLONTECH) for 18 h. Filters were washed under high stringency condition, i.e., four times in 0.1× SSC, 0.1% SDS at 24 °C, followed by two times at 50 °C in 0.1× SSC, 0.1% SDS.

In Situ Hybridization Studies—The kidneys of CD-1 mice were harvested after intracardiac catheter perfusion with 4% paraformaldehyde in phosphate-buffered saline, fixed at 4 °C for 3 h, and dehydrated in ethanol. The whole embryo of the CD-1 mouse at day 13 of gestation was also fixed with 4% paraformaldehyde in PBS. Tissues and embryos were embedded in paraffin; 4-µm-thick sections were prepared and

![Image](image_url)

**Table I**

Sequence similarity of mouse, rat, and human collectrin at nucleotide and amino acid levels

| Species       | Nucleotide level | Amino acid level |
|---------------|------------------|------------------|
| Mouse/Rat     | 94.2%            | 93.7             |
| Mouse/Human   | 86.9%            | 84.7             |
| Rat/Human     | 86.7%            | 85.1             |
| Mouse/Rat/Human | 84.3%         | 81.9             |

**Fig. 2.** Comparison of collectrin, ACE, and ACE2 protein sequences. A and B, multiple sequence alignment of ACE, ACE2, and collectrin. ACE (A31759), ACE2 (AF241254, AF291820), and human collectrin (AF229179) are aligned by DIALIGN based on a segment-to-segment comparison. The C terminus of ACE2 shares 47.8% identity with collectrin and ACE protein have similarity and shares 41.8% identity with ACE. A schematic drawing of the domain similarity is indicated (B). ACE consists of two homologous repeated domains of a catalytic site (gray box). ACE2 has one active site domain (gray box) and shows significant homology with the catalytic domain of ACE. Collectrin shows similarity with the C-terminal domain of ACE2; however, it lacks a catalytic domain for the dipeptidyl carboxypeptidase C, exon/intron boundaries of human collentrin and ACE2. Homology searches of the human genome sequences indicate that collectrin cDNA (AF229179) and ACE2 (AF241254, AF291820) match to the 159446-base BAC clone GS1-594A7 (AC003669, NT 001172), which is located on chromosome Xp22. Exon/intron structures are schematically indicated. The vertical bars in the BAC clone represent locations of exons and vertical lines on collectrin and ACE2 cDNAs indicate exon/intron boundaries. Four exons of collectrin cDNA and 18 exons of ACE2 cDNA are found on the BAC clone GS1-594A7.
mounted on RNase-free ProbeOn Plus glass slides (Fisher Scientific). Oligodeoxynucleotide spanning residues 370–398 of mouse collectrin cDNA (5'-CTCTTCCTGCAGCTGAAGTACAGTCGGCC-3', AS2; Fig. 1) linked to a 3'-biotinylated Bacterial HindIII tail [3'-T(A)9]2-BB[link]B(A)9-3', was synthesized and used as antisense probe (Research Genetics). Biotinylated poly(A) and poly(T) probes were used as positive and negative controls, respectively. In situ hybridization was performed using MicroProbe System (Fisher Scientific) and in situ sampler kit (Research Genetics, Huntsville, AL) following the manufacturer’s protocol. In brief, the tissue sections were rapidly dewaxed (AutoDewaxer), cleared with alcohol (AutoAlcohol), and rehydrated with a Tris-based buffer, pH 7.4 (Universal Buffer). The tissue sections were digested with a stable pepsin solution (0.5 mg/dl) for 3 min. The probes (17135) were applied for 5 min at 50 °C. The biotin-labeled hybrids were detected by horseradish peroxidase-linked streptavidin and diaminobenzidine reaction. The hybridizing signal of collectrin mRNA is observed in collecting tubules in the renal cortex (panels E and F) and medulla (panels G and H). No signal is observed on glomeruli or other segments of the tubules. Bar = 100 μm in panels E and G; bar = 50 μm in panels F and H.

**RESULTS**

**Isolation and Sequence Analysis of Mouse, Rat, and Human Collectrin**—By RACE-PCR using S1 and AS1 primers, cDNA containing the full-coding sequence of mouse collectrin was obtained (GenBankTM/EBI accession no. AF178085). The mouse collectrin cDNA included 1282 bp, and it contained a 666-bp open reading frame flanked by 5'- and 3'-untranslated regions. The potential initiation codon was located at position 87–89, and the open reading frame encoded 222 amino acid residues with a predicted molecular mass of ~25 kDa (Fig. 1A). The S1 and AS1 primer sequences matched the corresponding EST clones of both rat and human deposited in GenBankTM/EBI. Using these primers, 1181 bp of rat collectrin (AF178086) and 1345 bp of human collectrin (AF229179) cDNAs were also
isolated by RACE-PCR (Fig. 1B). A ClustalW multiple alignment indicates that the primary protein structure of mouse, rat, and human collectrin was highly conserved throughout the entire sequence (Fig. 1B). They shared 84.3% and 81.9% identity at the nucleotide and amino acid levels, respectively (Table I).

**Structural and Homology Analysis of Collectrin**—Kyte and Doolittle hydrophobicity/hydrophilicity plot analysis (12) predicted that the protein had two hydrophobic domains (Fig. 1C). The N-terminal hydrophobic domain (amino acids 1–14) was predicted as a signal sequence by von Heijne’s method (16), and a predicted cleavage site was located between amino acid residues 14 and 15. The second hydrophobic domain stretched between residues 142 and 164, and it seemed to have the characteristics of a transmembrane domain (SOSUI; Ref. 17). Thus, collectrin seemed to be a type 1a membrane protein. A mitochondrial targeting sequence, an endoplasmic reticulum retention signal, and a peroxisomal targeting signal were not predicted. An N-terminal mitochondrial targeting sequence has been identified for a single-domain member of the collectrin family (17), and thus it does not share the segments or domains of ACE.

The multiple sequence alignment of the three proteins ACE, ACE2, and collectrin is shown in Fig. 2B.

Homology searches through human genome sequences indicates that the human collectrin cDNA matches the 159446-base BAC clone GS1-594A7 (AC003669, NT 001172) localized to chromosome Xp22. Four exons were found on the BAC clone, and they corresponded to residues 142–1345 of the human collectrin cDNA (Fig. 2C). The exon(s) corresponding to nucleotides 1–141 of the human collectrin cDNA was not discovered in searches of human genome sequences deposited in GenBank™/EBI. The ACE2 gene was also located on BAC clone GS1-594A7, and the alignment between ACE2 mRNA and the BAC clone GS1-594A7 revealed that the ACE2 gene spanned 40 kilobases, comprised 18 exons, and was located ~26 kilobases from the collectrin gene.

Collectrin is a kidney-specific gene and is localized to the Kidney Collecting Ducts in Vivo—Tissue distribution of collectrin mRNA was investigated by Northern blot analyses using mouse, rat, and human collectrin cDNAs as the hybridization probes (Fig. 3, A–D). A single transcript of ~1.8 kilobases was observed exclusively in the kidney, and it was not detected in any other tissues. The transcript size was similar in mouse, rat, and human, and no alternative splicing variants were observed. To investigate the mRNA localization of collectrin, in situ hybridization was performed using CD-1 mouse kidney (Fig. 3, E–H). The hybridizing signals of collectrin mRNA were observed in collecting tubules in the renal cortex (Fig. 3, E and F) and medulla (Fig. 3, G and H), indicating the whole segment of collecting duct expressed collectrin mRNA. By in situ hybridization of the whole mouse embryo at 13 days of gestation, collectrin mRNA was not expressed in any other tissues except kidney (data not shown).

The localization of collectrin was further investigated. To test the specificity of rabbit anti-collectrin antibody, immunoprecipitation studies using M-1 and mIMCD-3 collecting duct cell lines and SV40 MES 13 glomerular mesangial cell line were performed. A ~32-kDa single band was observed in an anti-collectrin-immunoprecipitated protein fraction derived from M-1 and mIMCD-3 collecting duct cells, and the band was absent in SV40 MES 13 cells (Fig. 4). Preimmune sera also did not show a specific band corresponding to the size of collectrin.

The specificity of anti-collectrin antibody was further verified by competitive inhibition ELISA assay as described previously (data not shown) (13).

Collectrin was localized in the cytoplasm of collecting duct cells in the cortex (Fig. 5A) and medulla (Fig. 5C). High magnification micrographs revealed linear immunoreactivity along the luminal surface of collecting ducts (Fig. 5, B and D). The epithelial cells in the renal pelvis also expressed collectrin (Fig. 5C). The immunostaining of serial sections indicated that the distribution of collectrin (Fig. 5E) matched with aquaporin-2, which is the marker for collecting ducts (Fig. 5F). To further confirm the localization of collectrin, the avidin-biotin complex method was employed. Collectrin was expressed in the cytoplasm of collecting tubule in the cortex (Fig. 5G) and medulla.
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In this investigation, isolation and characterization of a novel renal-specific type 1α transmembrane glycoprotein, designated as collectrin, is described. Homology analysis indicates that collectrin has a long-ordered homology with ACE2, which that collectrin has a long-ordered homology with ACE2, which consists of only one catalytic domain (20). The ACE2 protein also has one active site domain and shares 41.8% identity with ACE. Although ACE is ubiquitously expressed in the vasculature, ACE2 is restricted to heart, kidney, and testis (8). In contrast to ACE and ACE2, collectrin lacks the metalloprotease catalytic domains, although it shares 47.8% identity with the non-catalytic extracellular, transmembrane, and intracellular domains of ACE2. Both ACE2 and collectrin revealed tissue-restricted expression in kidney. However, ACE2 is present throughout the endothelium and in proximal tubular epithelial cells, whereas collectrin is present in collecting duct epithelia only. Homology searches of human collectrin cDNA in the human genome indicate that it is localized to chromosome Xp22. Interestingly, ACE2 is localized in the same BAC clone that is close to the collectrin gene. Exon/intron organization and chromosome localization of ACE, ACE2, and collectrin suggests that these three genes evolved from a common ancestral gene.

The catalytic activity of ACE2 has been reported; removing the C-terminal Leu from Ang I and generating Ang1–9. In contrast, ACE cleaves the His-Leu dipeptide and converts Ang I to Ang II (Ang1–8; Ref. 8). A multiple sequence alignment indicates that the catalytic domain is apparently absent, and extensive searches for domain structures or consensus sequences have not revealed any additional information as yet. Clinical and experimental studies in animals have revealed that the ACE inhibitors or Ang II receptor antagonists decrease proteinuria and slow the progression of diabetic nephropathy and various forms of glomerulonephritis. The finding that the

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In this investigation, isolation and characterization of a novel renal-specific type 1α transmembrane glycoprotein, designated as collectrin, is described. Homology analysis indicates that collectrin has a long-ordered homology with ACE2, which is the first human homolog of ACE and was identified from 5′ sequencing of a human heart failure ventricle cDNA library (8). A renal and pulmonary splicing isoform of ACE (A31759) consists of two homologous repeats of catalytic domains, whereas the testicular isoform (S05238) consists of only one catalytic domain (20). The ACE2 protein also has one active site domain and shares 41.8% identity with ACE. Although ACE is ubiquitously expressed in the vasculature, ACE2 is restricted to heart, kidney, and testis (8). In contrast to ACE and ACE2, collectrin lacks the metalloprotease catalytic domains, although it shares 47.8% identity with the non-catalytic extracellular, transmembrane, and intracellular domains of ACE2. Both ACE2 and collectrin revealed tissue-restricted expression in kidney. However, ACE2 is present throughout the endothelium and in proximal tubular epithelial cells, whereas collectrin is present in collecting duct epithelia only. Homology searches of human collectrin cDNA in the human genome indicate that it is localized to chromosome Xp22. Interestingly, ACE2 is localized in the same BAC clone that is close to the collectrin gene. Exon/intron organization and chromosome localization of ACE, ACE2, and collectrin suggests that these three genes evolved from a common ancestral gene.

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systemic renin-angiotensin system (RAS) is not activated in most types of chronic renal disease has led to the suggestion that a local intrarenal RAS plays important roles in the relentless progression of renal disease. In this regard, it is interesting to note that renal tubular cells are capable of generating Ang II and Ang II type 1 (AT1) receptor proteins that are implicated in various kidney diseases. In Sprague-Dawley rats with subtotal nephrectomy, renin and Ang II are up-regulated and implicated in the pathogenesis of tubulo-interstitial fibrosis (21), as is the case in uninephrectomized Wistar-Kyoto rats, where ACE has been reported to be up-regulated in proximal tubules (22). Because Ang II is involved in renal cell growth and matrix production via the activation of AT1 receptor (23), Ang II may be responsible for the tubulo-interstitial lesions seen in this model. Along these lines it is conceivable that up-regulated expression of the angiotensinogen gene, as observed in proximal tubular cell line under high glucose conditions, may contribute to a certain degree to the progression of tubulo-interstitial lesions in diabetic nephropathy (24).

Studies reported in the literature suggest that intrarenal RAS, especially in the tubular cells, plays a critical role in the progression of interstitial tissue injury. The latter of course is certainly linked to the deterioration of renal functions in various kidney diseases. Conceivably, collectrin, which is homologous to ACE2 and up-regulated in the hypertrophic phase of the ablated kidney in 5/6 nephrectomized Sprague-Dawley rats, would be another key molecule that may be relevant to the renal tissue injury. In contrast to ACE and ACE2, collectrin does not contain dipeptidyl carboxypeptidase domains, and thus it may play a role in hypertrophic phase kidneys via other yet to be characterized mechanism(s) rather than via RAS activation. To explore such a role in the progression of tubulo-interstitial renal tissue injury, determination of the biological activity of collectrin will be the subject of future investigations.

Because the administration of RAS antagonists causes widespread structural and growth abnormalities in the kidney, the enhanced expression of RAS genes in fetal and early postnatal life seems to predict critical functions related to renal growth and development (25, 26). Actually, targeted inactivation of the angiotensinogen gene induced adulthood developmental abnormalities including vascular hypertrophy, focal tubular dropout with interstitial inflammatory infiltrates, and marked atrophy of the renal papilla (27, 28). This phenotype is also seen in mice that completely lack ACE (29, 30). Although mice lacking AT1A (31–34) receptor genes survive in normal numbers, and their renal morphology is reported to be normal with the exception of some minor abnormalities of the inner medulla and papilla, mice lacking both AT1A and AT1B genes do not develop a renal pelvis resulting in the buildup of urine and progressive kidney damage (35). Ang II and its receptor are transiently up-regulated at the renal outlet at birth and related to peristaltic movements of the pelvis to transfer 50-fold-increased urine,

**Fig. 6.** Northern blot analyses of mouse collectrin mRNA expressed in various developmental stages of mouse kidney. 20 μg of total RNAs from mouse kidneys of various developmental stages were subjected to 2.2 M formaldehyde, 1% agarose-gel electrophoresis and capillary transferred to nylon membranes. The membranes were hybridized with [α-32P]dCTP-radiolabeled mouse collectrin cDNA. The membrane was reprobed with GAPDH (1 × 106 cpm/ml). mRNA signal of collectrin is detectable at 13-day of gestation and its expression is progressively increased during the later stages of gestation extending into the neonatal periods. The mRNA expression is then much reduced in adult life of the CD-1 mouse. Lanes 13d and 17d, renal total RNA isolated from 13-day- and 17-day-old mouse fetuses. Lanes NB, 1W, 2W, and AD represent newborn, 1-week-, 2-week-, and 8-week-old mice renal total RNAs, respectively.

**Fig. 7.** Immunohistochemical localization of collectrin in various developmental stages of mouse kidney. By immunofluorescence study, collectrin is observed in the ureteric bud branches at day 13 of gestation (13d) of the CD-1 mouse (A and B). Immunoreactivity of collectrin is increased in day 17 of gestation (17d), and it is expressed in the developing collecting ducts (C and D). After birth, the enhanced expression of collectrin is maintained during postnatal periods, i.e. newborn (NB, E, and F), 1-week-old (1W, G, and H), and 2-week-old (2W, I, and J) mice. Collectrin is expressed in the collecting ducts in the cortex, medulla, and epithelial cells lining the pelvis. In 8-week-old adult kidneys (AD, K, and L), immunoreactivity of collectrin is rather decreased. Bar = 500 μm in A, C, E, G, I, and K; bar = 100 μm in B, D, F, H, J, and L.
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Compared with embryonic life (35). The evidence that collectrin up-regulates the perinatal period and is expressed in collecting ducts and epithelia of renal pelvis suggests that collectrin may have a role in the development of collecting ducts and renal pelvis. Because collectrin has homology with ACE2, a novel member of RAS, collectrin may be involved in the functions related to the RAS, e.g., the urinary peristaltic machinery during the perinatal period.

The identification of collectrin, a novel homolog of ACE2, adds complexity to the RAS and may facilitate the discovery of novel role(s) of the RAS in the pathophysiology of renal collecting ducts and pelvises. At present, the obvious questions that one can raise would be why collectrin is lacking catalytic domains compared with embryonic life (35). The evidence that collectrin up-regulates the perinatal period and is expressed in collecting ducts and epithelia of renal pelvis suggests that collectrin may have a role in the development of collecting ducts and renal pelvis. Because collectrin has homology with ACE2, a novel member of RAS, collectrin may be involved in the functions related to the RAS, e.g., the urinary peristaltic machinery during the perinatal period.

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