BMC Physiology

BioMed Central

Research article

Differential expression of E-cadherin, N-cadherin and beta-catenin in proximal and distal segments of the rat nephron.

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Published: 17 May 2004

BMC Physiology 2004, **4**:10 doi:10.1186/1472-6793-4-10

Received: 02 March 2004
Accepted: 17 May 2004

This article is available from: http://www.biomedcentral.com/1472-6793/4/10

Abstract

Background: The classical cadherins such as E- and N-cadherin are Ca^{2+}-dependent cell adhesion molecules that play important roles in the development and maintenance of renal epithelial polarity. Recent studies have shown that a variety of cadherins are present in the kidney and are differentially expressed in various segments of the nephron. However, the interpretation of these findings has been complicated by the fact that the various studies focused on different panels of cadherins and utilized different species. Moreover, since only a few of the previous studies focused on the rat, information regarding the expression and localization of renal cadherins in this important species is lacking. In the present study, we have employed dual immunofluorescent labeling procedures that utilized specific antibodies against either E- or N-cadherin, along with antibodies that target markers for specific nephron segments, to characterize the patterns of cadherin expression in frozen sections of adult rat kidney.

Results: The results showed that N-cadherin is the predominant cadherin in the proximal tubule, but is essentially absent in other nephron segments. By contrast, E-cadherin is abundant in the distal tubule, collecting duct and most medullary segments, but is present only at very low levels in the proximal tubule. Additional results revealed different patterns of N-cadherin labeling along various segments of the proximal tubule. The S1 and S2 segments exhibit a fine threadlike pattern of labeling at the apical cell surface, whereas the S3 segment show intense labeling at the lateral cell-cell contacts.

Conclusions: These results indicate that E- and N-cadherin are differentially expressed in the proximal and distal tubules of rat kidney and they raise the possibility that differences in cadherin expression and localization may contribute to the differences in the susceptibility of various nephron segments to renal pathology or nephrotoxic injury.

Background

The tubular segments of the nephron can be thought of as a series of functionally distinct units each having unique permeability characteristics and fluid and electrolyte transport capabilities. The specific permeability and transport properties of the individual nephron segments are determined by the general cytoarchitecture of the epithelial cells and also by the manner in which the cells interact with each other [1]. These cell-cell interactions involve specialized junctional complexes that are necessary for the restriction of permeability, the establishment of epithelial polarity and the normal transport of materials across the

cell monolayer (for reviews see [1-6]). These junctional complexes include: adherens junctions (zonulae adherens), occluding junctions (zonulae occludens or tight junctions), desmosomes and gap junctions. These complexes are composed of specific cell adhesion molecules and their associated scaffolding proteins that link the complexes to the cytoskeletal elements of the individual cells [1,5,7-9].

Of the many molecules that have been shown to be involved in renal epithelial cell-cell adhesion, the cadherins family of Ca^{2+}-dependent cell adhesion molecules are among the most important. While over 50 cadherins have been described, the best characterized are the classical (Type I) cadherins such as E-cadherin and N-cadherin (for reviews see [10,11]). These classical cadherins are integral, transmembrane proteins that are usually localized at the adherens junctions of epithelial cells [9,12-14]. The extracellular domain of the cadherin contains the Ca^{2+}-binding sites, as well as the adhesive regions of the molecule. The intracellular domain is bound to β-catenin which is bound to α-catenin, which in turn links the entire complex to the actin cytoskeleton [8,9,15-17]. In this context, the cadherin/catenin complex serves as a key structural component of adherens-type junctions. In addition, β-catenin functions as a component of the wingless or Wnt nuclear signaling pathway and plays an important role in the regulation of gene expression (for reviews see [18-21]).

Studies over the past 19 years have shown that a variety of cadherins, including E-, N-, P-, K-, R-, OB-, VE- and Ksp-cadherin are present in the kidney [22-32]. Several of these cadherins, such as OB-, R- and K-cadherin, are transiently expressed during different stages of development [29-31,33]. In adult kidney the most abundant cadherins appear to be the classical cadherins, N-, and E-cadherin [23,24,34-36], along with an atypical kidney-specific cadherin known as Ksp-cadherin [26,37]. The latter molecule differs from the classical cadherins in that it lacks the cytoplasmic catenin-binding domain [26,38,39]. At present, the functional significance of this atypical cadherin is unclear.

In terms of maintaining cell-cell adhesion along the nephron, the classical cadherins appear to be the key players, and a growing volume of evidence indicates that these classical cadherins are differentially expressed in various segments of the nephron. However, this issue is complicated by the fact that the various studies have focused on different panels of cadherins and utilized different species. In the first comprehensive studies to map the distribution of renal cadherins, Nouwen et al. [23] and Tani et al. [25] showed that in human kidney, N-cadherin is the predominant cadherin in proximal tubule, whereas E-cadherin is predominant in the distal tubule and other nephron segments. In a very elegant study of mouse kidney, Piepenhagen et al. [24] showed that E-cadherin is abundantly expressed in most segments of the nephron, including the proximal tubule, but they made no mention of N-cadherin. Cho et al. [29] also reported that E-cadherin is present in the proximal and distal tubules of newborn mice, but they, too, made no mention of N-cadherin. However, several more recent studies have shown that N-cadherin is present in the proximal tubule of the rat and mouse [34,35,40,41]. In a recent study examining the effects of the nephrotoxic metal Cd^{2+} on cadherin localization in rat kidney, we also observed that N-cadherin is present in the proximal tubule but is not expressed in other nephron segments [36]. In addition, we noticed what appeared to be different patterns of N-cadherin labeling along various segments of the proximal tubule; the S1 and S2 segments (proximal convoluted tubule) exhibited a fine, thread-like pattern of labeling near the apical cell surface, whereas the S3 segment (proximal straight tubule) showed intense labeling at the lateral cell-cell contacts. However, the specific identity of these tubular segments in that study was somewhat equivocal because their identification was based primarily on their general morphology and their location in sagittal cryosections. In the present study, we have employed dual immunofluorescence labeling procedures that utilized specific antibodies against either E- or N-cadherin, along with antibodies that target markers for specific nephron segments, to characterize the patterns of cadherin expression in cryosections of adult rat kidney. The results show that N-cadherin is, in fact, the predominant cadherin in the proximal tubule and that the convoluted and straight segments of the proximal tubule exhibit different patterns of N-cadherin labeling. By contrast, E-cadherin is abundant in the distal tubule, collecting duct and most medullary segments, but is present only at very low levels in the proximal tubule. The localization of the cadherin-binding protein β-catenin parallels that of both N- and E-cadherin.

Results

Results of dual labeling experiments showed that E-cadherin and N-cadherin are differentially localized in the kidney. Figure 1 shows the dual labeling of E- and N-cadherin in a field from the outer cortex. Note that the green E-cadherin labeling is concentrated at the lateral cell-cell contacts in a specific subpopulation of tubules. By contrast, the red N-cadherin labeling is concentrated in a different subpopulation of tubules. Moreover, the pattern of N-cadherin labeling differs from that of E-cadherin. The N-cadherin-labeled tubules exhibit a diffuse pattern of labeling on the basolateral cell surface and a fine, thread-like band of labeling near the apical cell surface. From their location and general morphology, the E-cadherin labeled structures were tentatively identified as mainly

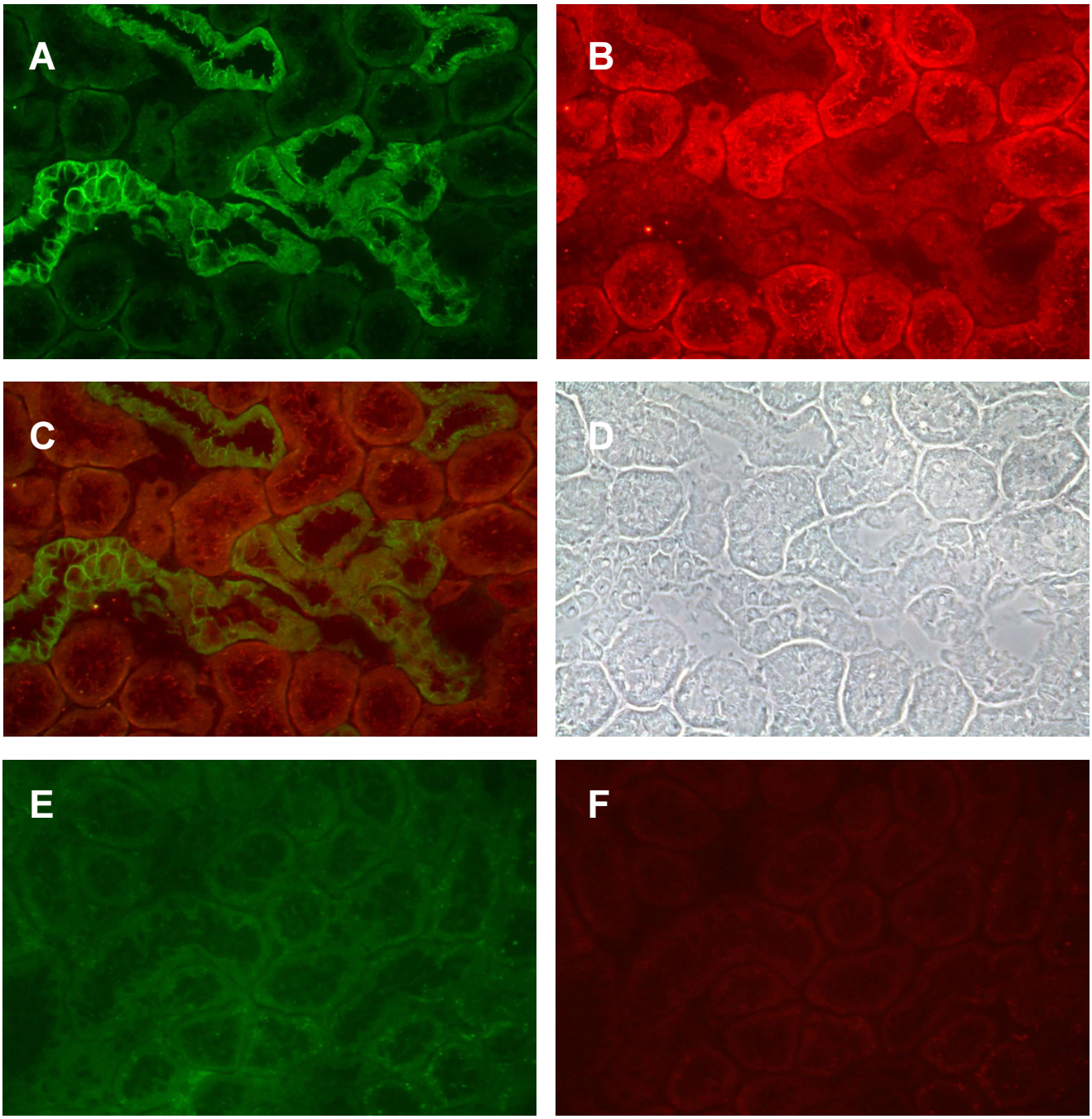

Figure 1
Dual labeling of E-cadherin and N-cadherin in the outer cortex. Samples were processed for the dual labeling of E-cadherin and N-cadherin as described in the Methods and then viewed using a 40× objective. A: E-cadherin; B: N-cadherin; C: Overlay of images A and B; D: Phase contrast image of the same field; E and F show the lack of E- and N-cadherin labeling in a control sample that was incubated without the primary antibodies. Original magnification = 174×.

distal tubules and collecting ducts, whereas the N-cadherin labeled tubules were tentatively identified as the S1 and S2 segments of the proximal tubule (i.e., proximal convoluted tubule). It should be noted that neither E- nor N-cadherin was detected in the glomeruli (not shown). As may be seen in the photos of the controls, blank samples

that were incubated in the presence of non-immune serum from the species in which the primary antibodies were generated showed a complete absence of labeling.

Figure 2 shows the dual labeling of E- and N-cadherin in a field from the inner cortex, near the outer stripe of the medulla. As in the outer cortex, the E-cadherin and N-cadherin labeling are concentrated in different populations of the tubules. The pattern of E-cadherin labeling in this region is also similar to that in the outer cortex, with the labeling concentrated at the lateral cell-cell contacts. Based on their morphology and location, these E-cadherin labeled structures most likely are distal tubules, collecting ducts and possibly portions of the loop of Henle. By contrast, the N-cadherin labeling is concentrated in a different population of tubules. Based on their location and morphology, these N-cadherin labeled structures were tentatively identified as the S3 segment of the proximal tubule (proximal straight tubule). Interestingly, the pattern of N-cadherin labeling in the inner cortex is somewhat different from that observed in the outer cortex. The N-cadherin labeling in the inner cortex is highly concentrated along the basolateral cell surface and at the lateral cell-cell contacts. None of the apical threadlike labeling that was observed in the outer cortex is evident in this region.

Figure 3 shows the dual labeling of E- and N-cadherin in a typical field from the renal medulla. Note that most of the tubular structures show intense E-cadherin labeling that is concentrated at the lateral cell-cell contacts. However, no N-cadherin labeling is evident in the medulla.

These studies examining the dual labeling of E- and N-cadherin indicated that E-cadherin is the predominant cadherin in most segments of the rat nephron, but that N-cadherin is the predominant cadherin in a subpopulation of tubules that were tentatively identified as proximal tubules. To verify this observation, we employed another dual labeling procedure to visualize N-cadherin and the proximal tubule marker protein aquaporin 1 [42-45] in the same samples. Panels A-B in Figure 4 show the dual labeling of N-cadherin and aquaporin 1 in a field from the outer cortex. Note that diffuse aquaporin 1 labeling is evident on the surface of the cells in some tubules, but not others (Panel A). The same tubules that exhibit the aquaporin 1 labeling are the ones that also exhibit N-cadherin labeling (Panel B and image overlay C). Panels E and F show a similar field viewed at higher magnification. This higher magnification gives an excellent image of the typical pattern of N-cadherin labeling in the outer cortex. Note the fine, thread-like pattern of labeling at the apical cell-cell contacts and the more diffuse labeling along the basement membrane and basal cell surface. In light of their location, general morphology and expression of aquaporin 1, these N-cadherin labeled structures in the outer cortex most likely are the S1/S2 segments of the proximal tubule (proximal convoluted tubule).

Figure 5 shows the dual labeling of N-cadherin and aquaporin 1 in a field from the inner cortex. As may be seen in Panel A, aquaporin 1 labeling is evident on the surface of the cells in some, but not all, tubules. These aquaporin 1-labeled tubules are the same tubules that show intense N-cadherin labeling (Panel B and image overlay C), and they almost certainly represent the S3 segments of the proximal tubule (proximal straight tubule). Panels E and F show the aquaporin 1 and N-cadherin labeling in a similar field at higher magnification. Note that the pattern of N-cadherin labeling in this segment is quite different form that in the S1/S2 segments (compare with Figure 4B). Here, the labeling is concentrated at the lateral cell-cell contacts, and there is no labeling on the apical cell surface.

In order to further identify the cortical structures that express high levels of E-cadherin, we employed another dual labeling procedure to visualize E-cadherin and either aquaporin 1 or aquaporin 2 in the same samples. The latter molecule is a vasopressin-sensitive water channel protein that is found at high levels in the principal cells of the collecting duct, but is essentially absent in other nephron segments [44,45]. Panels A and B in Figure 6 show the dual labeling of proximal tubule marker aquaporin 1 and E-cadherin in a field from the outer cortex. As may be seen, the tubules that exhibit the most intense aquaporin 1 labeling (Panel A) show only weak E-cadherin labeling (Panel B). Conversely, the tubules that show the most intense E-cadherin labeling show no labeling for aquaporin 1, indicating that the E-cadherin labeled structures are not proximal tubules. Panels C and D show the dual labeling of the collecting duct marker aquaporin 2 (C) and E-cadherin (D) in a similar field from the outer cortex. As may be seen in panel C, the aquaporin 2 labeling is confined to a few, smaller, but well defined tubular structures in the center and lower right regions of the field. These tubules, which are most likely collecting ducts (CD), also exhibit prominent E-cadherin labeling at the lateral cell-cell contacts (Panel D). In addition, intense E-cadherin labeling is present in other tubules in the upper right region of the field that do not express aquaporin 2. Based on their location and morphology, these are most likely distal tubules (DT). Panels E and F show higher magnification images of the patterns of E-cadherin labeling in a collecting duct and distal tubule respectively.

In an additional component of this study, we examined the localization of the cadherin-binding and nuclear signaling protein β-catenin in relation to that of E-cadherin and N-cadherin. Panels A and B in figure 7 show the

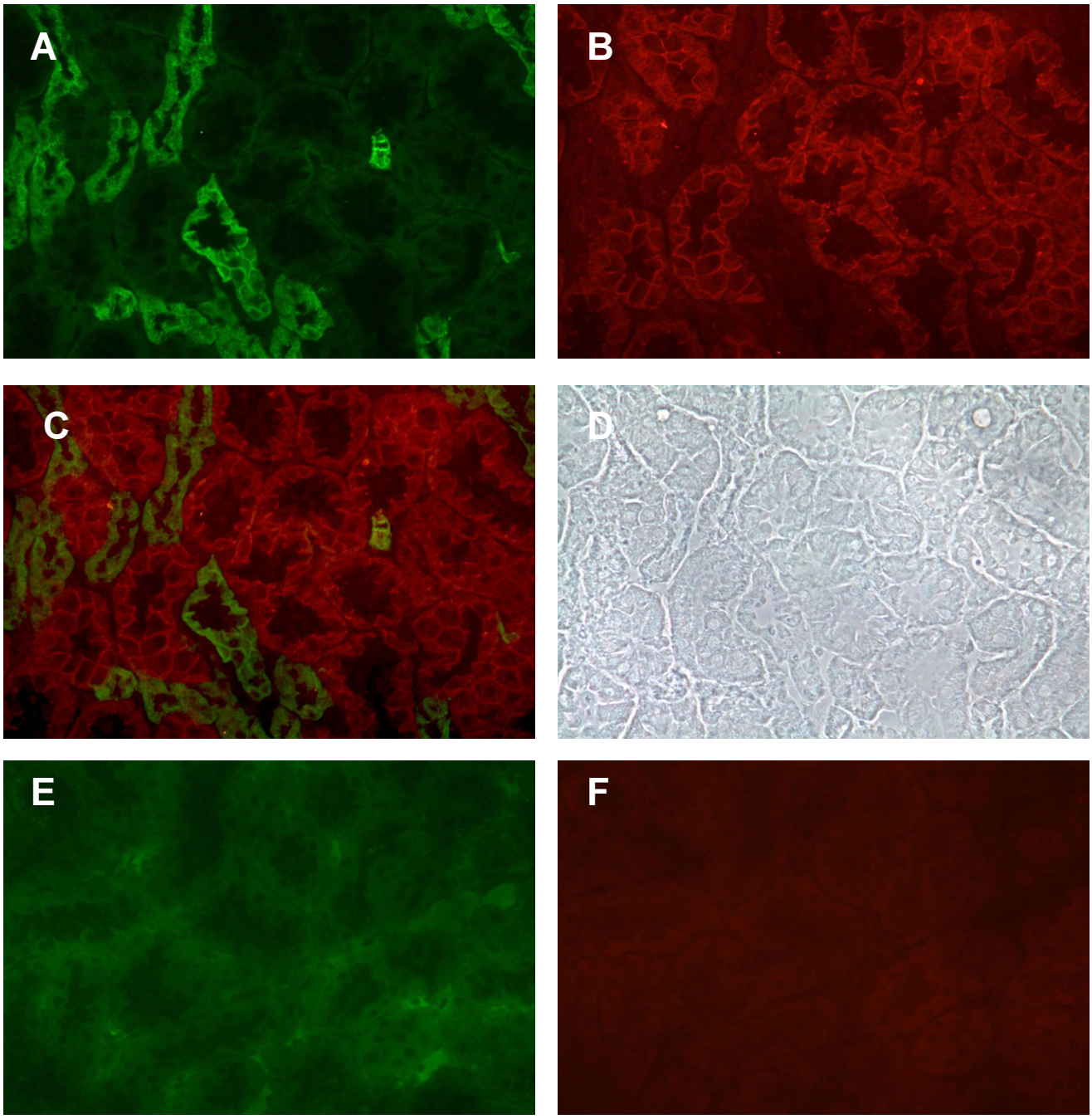

Figure 2
Dual labeling of E-cadherin and N-cadherin in the inner cortex. Samples were processed for the dual labeling of E-cadherin and N-cadherin as described in the Methods and then viewed using a 40× objective. A: E-cadherin; B: N-cadherin; C: Overlay of images A and B; D: Phase contrast image of the same field; E and F show the lack of E- and N-cadherin labeling in a control sample that was incubated without the primary antibodies. Original magnification = 174×.

dual labeling of β-catenin (A) and N-cadherin (B), and panels C and D show the dual labeling of β-catenin (C) and E-cadherin (D) in fields from the outer cortex. As may be seen, β-catenin labeling is evident in essentially all of the tubules (A and C) and is colocalized with both N-cadherin (B) and E-cadherin (D). Panels E-H show these same molecules in fields from the inner cortex. Again, β-catenin labeling is present in all tubules (E and G) where

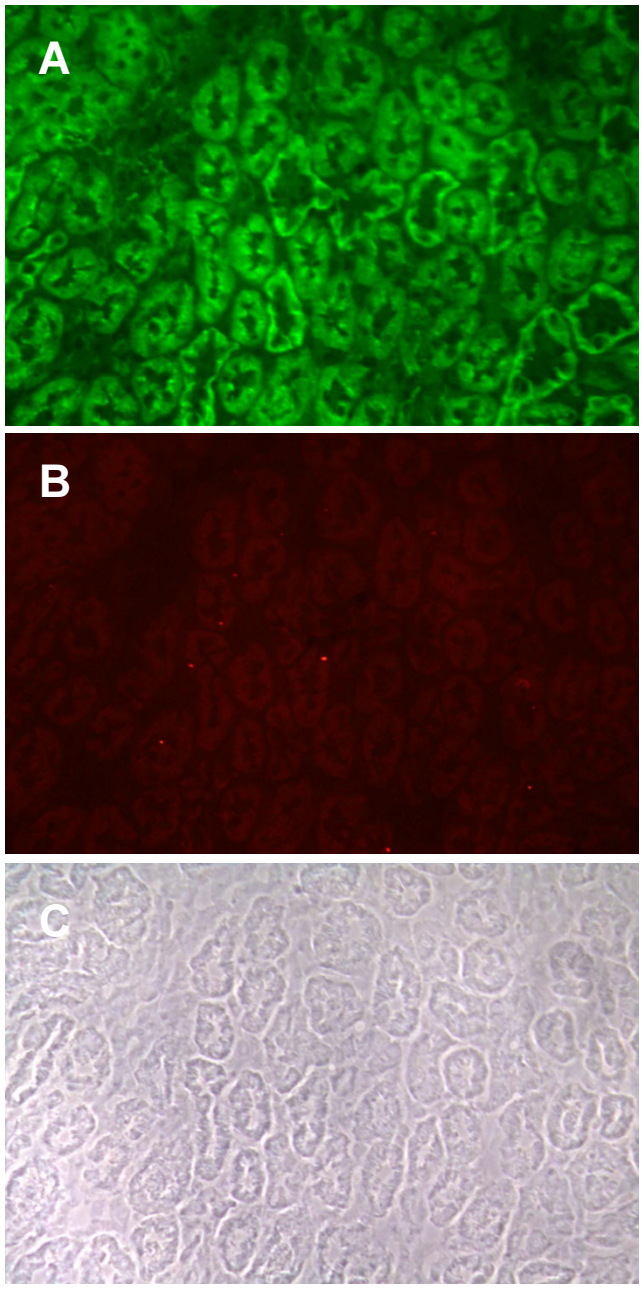

Figure 3
Dual labeling of E-cadherin and N-cadherin in the renal medulla. Samples were processed for the dual labeling of E-cadherin and N-cadherin as described in the Methods and then viewed using a 40× objective. A: E-cadherin; B: N-cadherin; C: Phase contrast image of the same field. Original magnification = 174×.

it is colocalized with both N-cadherin (F) and E-cadherin (H). Interestingly, the β-catenin labeling in the various segments of the proximal tubule exhibits the same patterns of labeling as N-cadherin. This is especially evident in the photos in Figure 8, which show higher magnification images of the dual labeling of N-cadherin and β-catenin in the S1/S2 segments and the S3 segment of the proximal tubule. In the S1/S2 segments (Panels A and B), both molecules show diffuse labeling along the basement membrane and the fine, thread-like labeling near the apical cell surface. In the S3 segment (Panels E and F) both molecules show intense labeling at the basolateral cell surface and the lateral cell-cell contacts.

To the best of our knowledge, the apical thread-like pattern of N-cadherin labeling that we observed in the S1/S2 segments of the proximal tubule has not been described previously in rat kidney. To further characterize this unusual pattern of labeling, we utilized a deconvolution technique to examine the labeling in a series of optical planes through a single section. The images were then used to construct a video clip showing the N-cadherin labeling as the plane of focus was moved through the section. The serial images in the video clip can be viewed as an 1. Note that as the plane of focus moves through the sample, the fine thread-like labeling can be seen to be especially concentrated at the lateral contacts between the epithelial cells just below the apical surface.

Discussion

Previous studies have shown that a variety of cadherins are present in the kidney, where they serve critical roles in establishing epithelial polarity and regulating barrier function [1,28,46]. In addition, recent studies suggest that alternations in the expression and function of renal cadherins and/or β-catenin may be associated with a variety of pathologic conditions including: glomerulonephritis [47], polycystic kidney disease [48-52], renal ischemic injury [53,54], renal carcinogenesis [55,56] and metal nephrotoxicity [34,36,40,57]. Thus, an understanding of the specific distribution and function of cadherins in the kidney may provide new insights into renal function and pathophysiology. While numerous studies in the literature have shown that various cadherins are differentially expressed along the nephron, the interpretation of the findings has been complicated by the fact that the various studies focused on different panels of cadherins and utilized different species. Moreover, since only a few of the previous studies focused on the rat, information regarding the expression and localization of renal cadherins in this important species is lacking.

The results of the present study show that E-cadherin and N-cadherin are differentially expressed along the adult rat nephron. E-cadherin is abundant in the distal tubule, collecting duct and most other nephron segments. By contrast, N-cadherin is abundant in the proximal tubule but is not expressed in other nephron segments. Not sur-

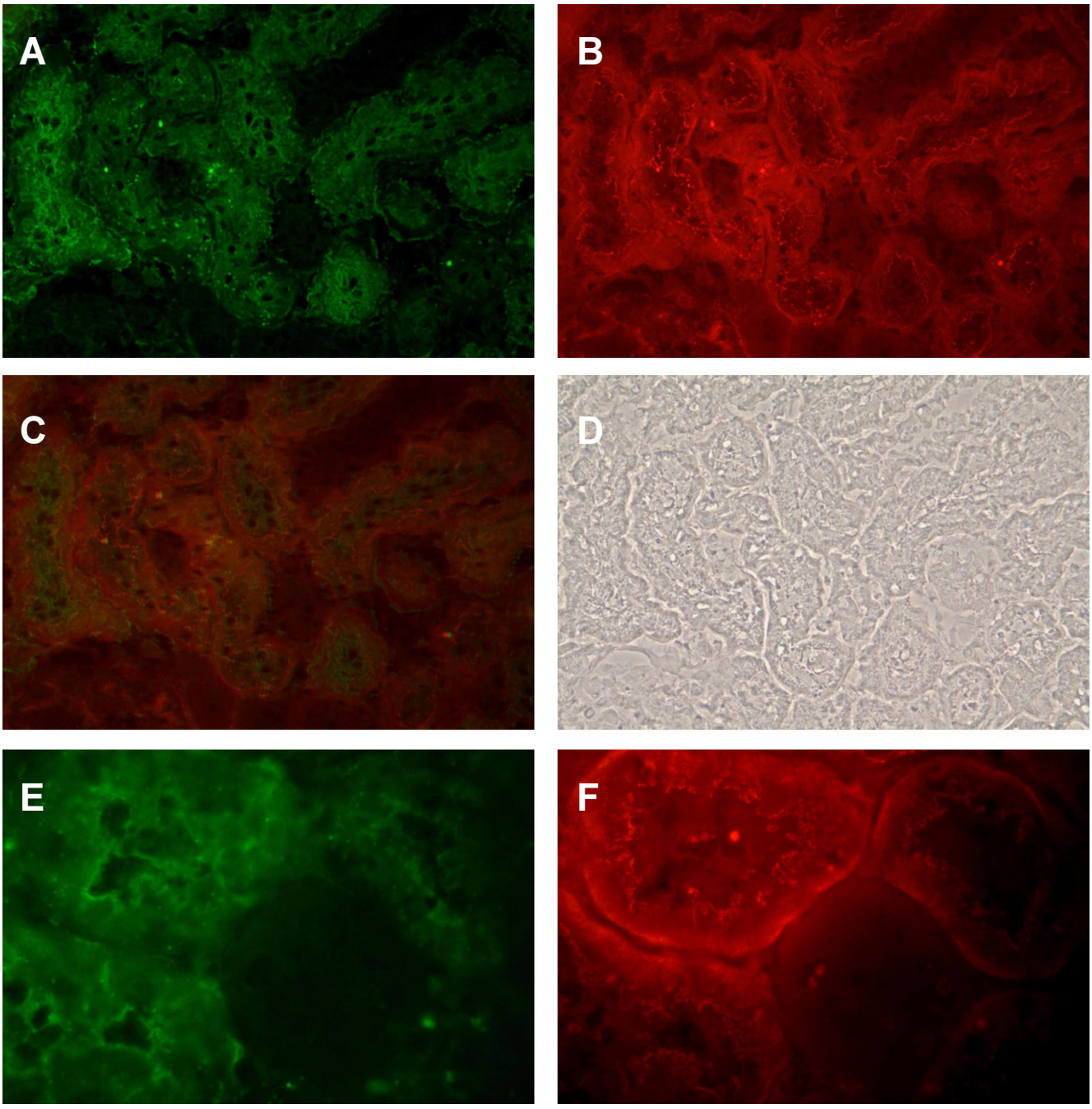

Figure 4
Dual labeling of aquaporin 1 and N-cadherin in the outer cortex. Samples were processed for the dual labeling of the proximal tubule marker aquaporin 1 and N-cadherin as described in the Methods and then viewed using a 40× or a 100× objective. Panels A-D show lower power images of the same field. A: Aquaporin 1; B: N-cadherin; C: Overlay of images A and B; D: Phase contrast image of the same field. Panels E and F show the dual labeling in a different field viewed at higher magnification. E: Aquaporin 1; F: N-cadherin. Original magnification = 174× (Panels A-D) and 435× (Panels E and F).

prisingly, the distribution of the cadherin-binding protein β-catenin closely parallels that of both E- and N-cadherin.

The findings that N-cadherin is present at much higher levels than E-cadherin in the proximal tubule of the rat kidney was somewhat surprising in light of the elegant

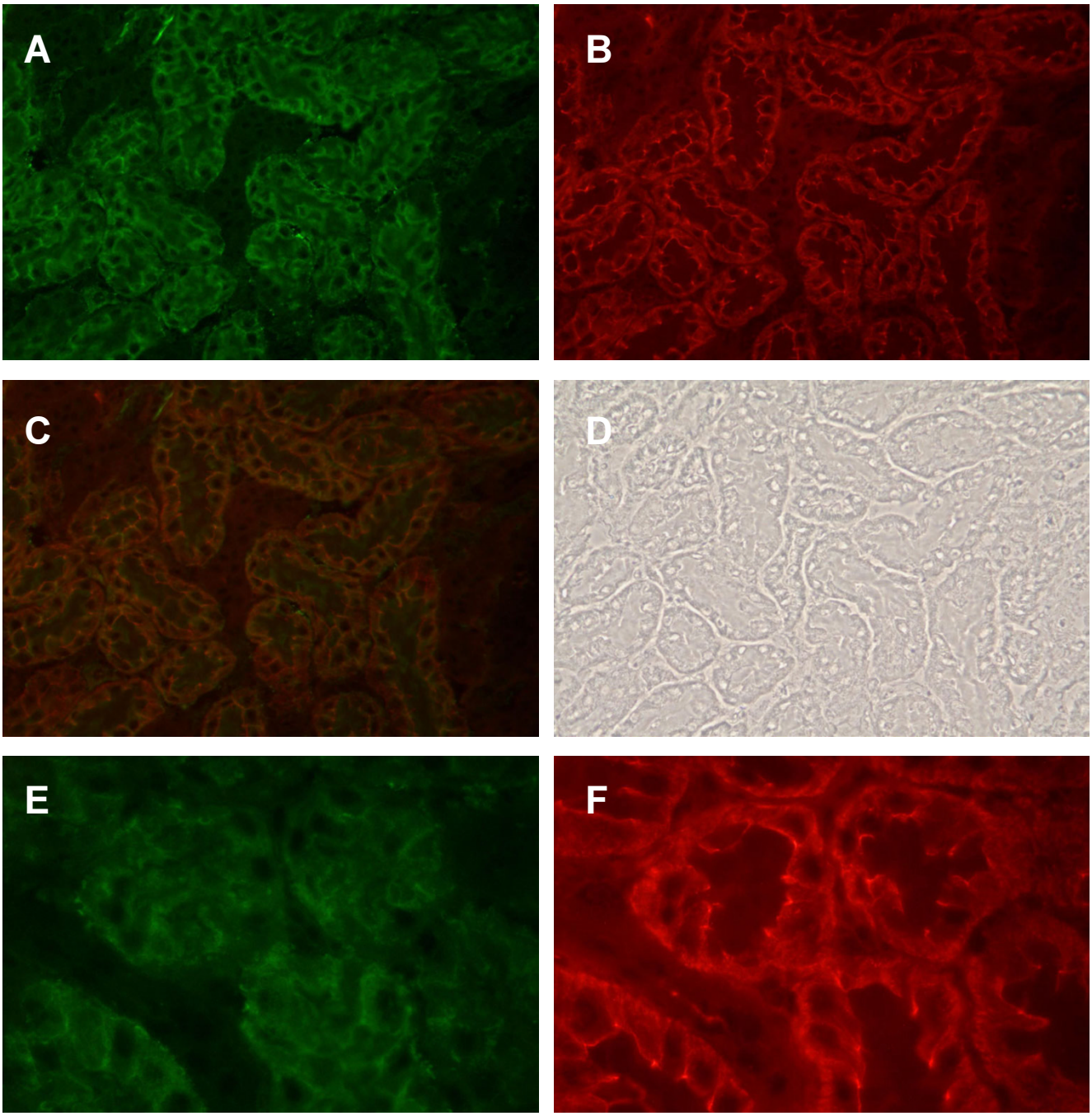

Figure 5
Dual labeling of Aquaporin 1 and N-cadherin in the inner cortex. Samples were processed for the dual labeling of the proximal tubule marker aquaporin 1 and N-cadherin as described in the Methods and then viewed using a 40× or a 100× objective. Panels A-D show lower images of the same field. A: Aquaporin 1; B: N-cadherin; C: Overlay of images A and B; D: Phase contrast image of the same field. Panels E and F show the dual labeling in a different field viewed at higher magnification. E: Aquaporin 1; F: N-cadherin. Original magnification = 174× (Panels A-D) and 435× (Panels E and F).

studies by Piepenhagen et al. [24] and Cho et al. [29] showing that E-cadherin is abundant in the proximal tubule of mouse kidney, and the fact that proximal tubule derived epithelial cell lines from a variety of species primarily express E-cadherin. However, our findings are

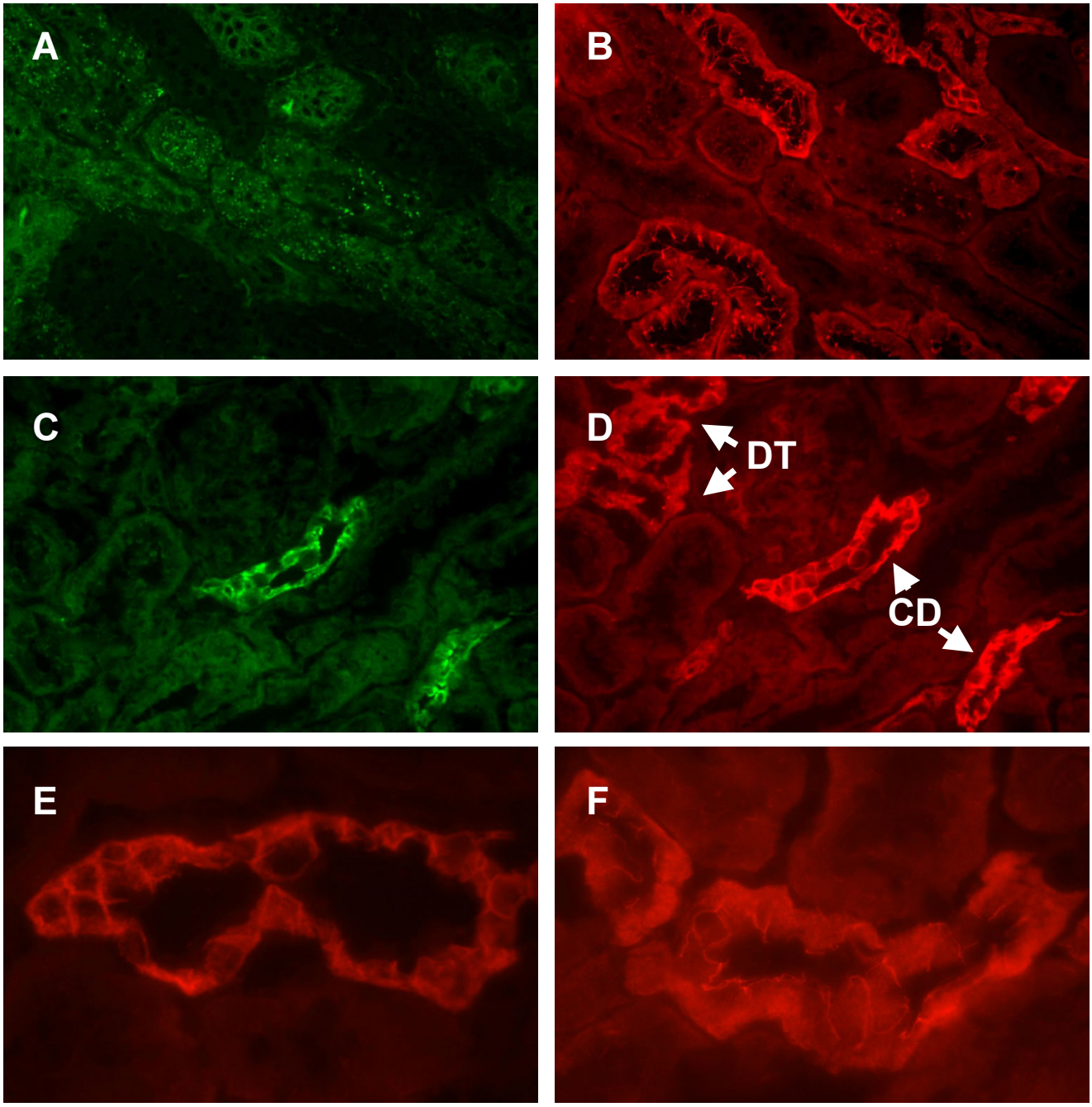

Figure 6
Dual labeling of E-cadherin and aquaporin 1 or aquaporin 2 in the outer cortex. Samples were processed for the dual labeling of E-cadherin and either aquaporin 1 or aquaporin 2 as described in the Methods and viewed using either a 40× or a 100× objective. Panels A and B show dual labeling of aquaporin 1 (A) and E-cadherin (B) in the same field. Panels C and D show the dual labeling of aquaporin 2 (C) and E-cadherin (D). CD denotes collecting ducts and DT denotes distal tubules. (E) E-cadherin labeling in a collecting duct. (F) E-cadherin labeling in a distal tubule. Original magnification = 174× (Panels A-D) and 435× (Panels E and F).

consistent with the recent reports by Leussink et al. [34] and Parrish et al. [35] showing that N-cadherin is expressed in the proximal tubule of rat kidney.

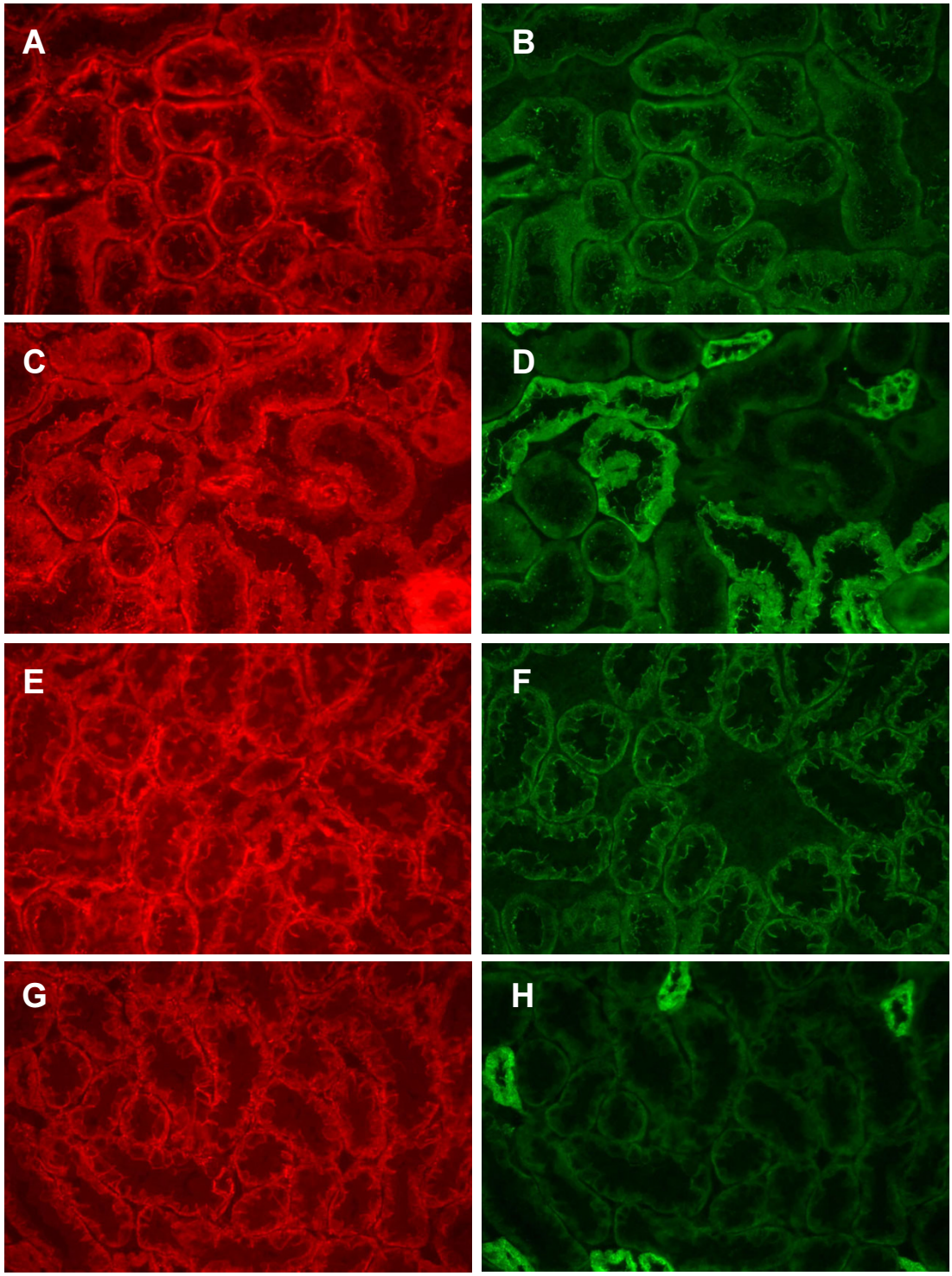

Figure 7
β-catenin is colocalized with both N-cadherin and E-cadherin. Samples were processed for the dual labeling of β-catenin and either N- or E-cadherin as described in the methods and viewed using a 40× objective. Panels A and B show the dual labeling of β-catenin (A) and N-cadherin (B) in a field from the outer cortex whereas panels C and D show the dual labeling of β-catenin (C) and E-cadherin (D) in a similar field. Panels E-F show the same molecules in fields from the inner cortex. E and F: dual labeling of β-catenin (E) and N-cadherin (F); G and H dual labeling of β-catenin (G) and E-cadherin (H). Original magnification = 174×.

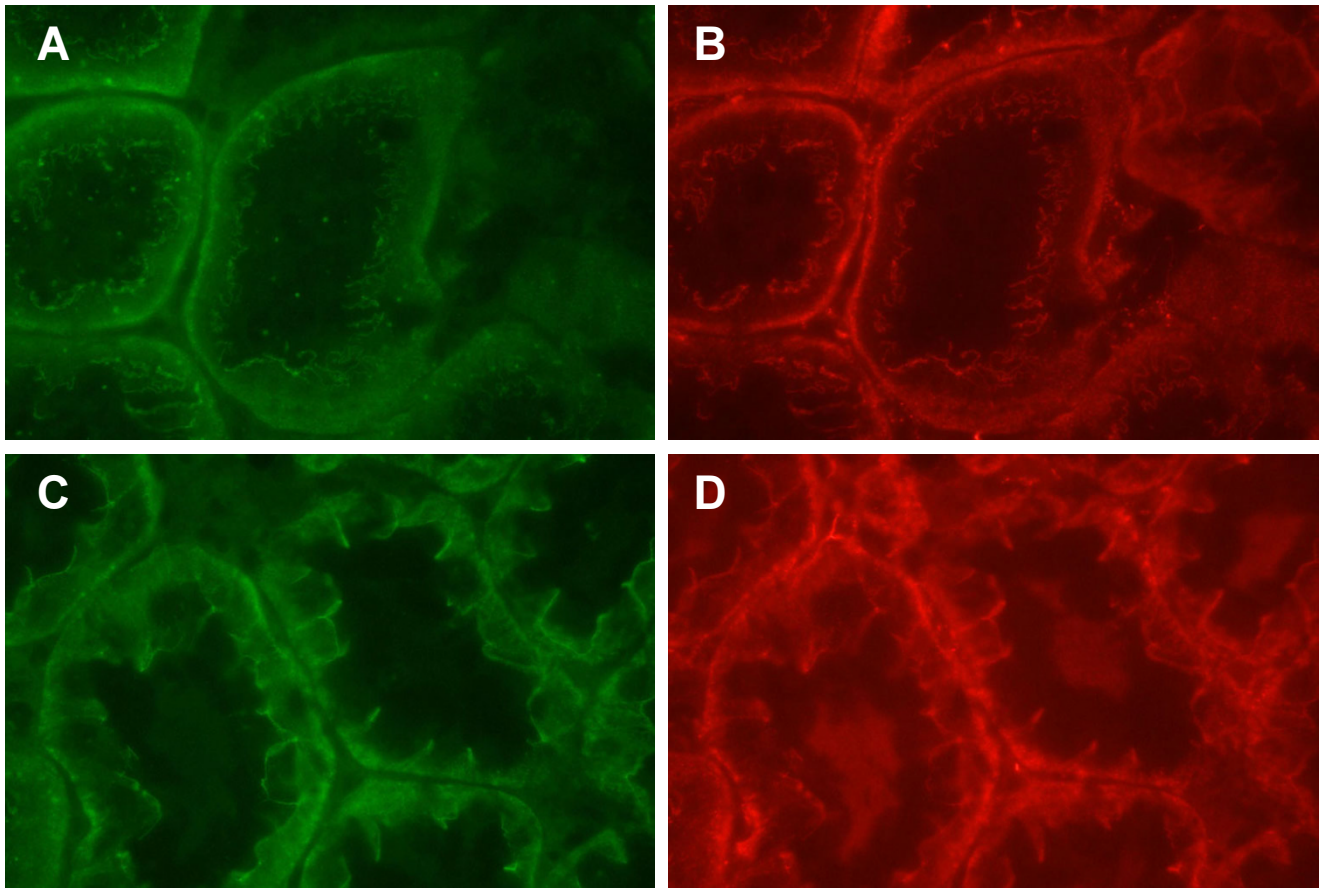

Figure 8
Dual labeling of N-cadherin and β-catenin in the proximal tubule. Panels A and B show that dual labeling of N-cadherin (A) and β-catenin (B) in the S1/S2 segment of the proximal tubule, whereas panels C and D show the dual labeling of N-cadherin (C) and β-catenin (D) in the S3 segment of the proximal tubule. Original magnification = 435×.

One of the more remarkable findings in the present study is that the pattern of N-cadherin localization varies markedly along the different segments of the proximal tubule. The S1/S2 segments of the proximal tubule exhibit a diffuse pattern of labeling along the basolateral cell surface and a fine-thread-like pattern of labeling at the cell contacts near the apical cell surface. By contrast, the N-cadherin labeling in the S3 segment is highly concentrated at the basolateral cell surface and the lateral cell-cell contacts. The pattern of N-cadherin labeling that we observed in the S3 segment is similar to that reported for the localization of other classical cadherins in renal tubules [24,29,34]. The apical, thread-like labeling, the pattern of N-cadherin that we observed in the S1/S2 segments of the proximal tubule is in many ways similar to the pattern reported by Nouwen, et al. [23] in their study of human kidney. It is also noteworthy that in our study, the pattern of β-catenin labeling in the proximal tubule closely paralleled that of N-cadherin. In S1/S2 segments both molecules exhibited the fine thread-like labeling near apical cell surface and in the S3 segment the labeling of both molecules was concentrated at the lateral cell-cell contacts. Interestingly, these different patterns of N-cadherin and β-catenin labeling that we observed in the various segments of the rat proximal tubule resemble in many respects the patterns of β-catenin labeling in the mouse proximal tubule described by Piepenhagen and Nelson [58].

At present, the significance of these differences in the patterns of N-cadherin localization in the various segments of the proximal tubule is unclear. However, since the classical cadherins such as N-cadherin are usually localized at the adherens junction of epithelial cells, the different patterns of N-cadherin labeling may reflect the differences in cell-cell interdigitations and the ultrastructure of the adherens junctions in various segments of the proximal tubule. Such cell-cell interdigitations are much more abundant in

the S1 and S2 segments of the proximal tubule than in the S3 segment M [59,60].

In light of the recent reports that alterations in renal cadherins may be associated with nephrotoxic injury, the finding that the levels and patterns of cadherin expression vary markedly along the nephron could also have important implications regarding the susceptibility of the various nephron segments to toxic injury. For example, the proximal tubule which expresses moderate levels of N-cadherin is especially sensitive to injury by nephrotoxic metals such as Cd^{2+}, Hg^{2+} and Bi^{2+}. Several recent reports indicate that the nephrotoxic effects of these metals are associated with alterations in the localization of N-cadherin in the proximal tubule [34,36,40]. By contrast, other segments of the nephron that express higher levels of E-cadherin are relatively resistant to injury by these metals. While the specific relationship between these metal-induced changes in cadherin localization and alterations in renal function are unclear, this would seem to be an interesting area for further research.

Conclusions

The results of the present studies show that N-cadherin and E-cadherin are differentially expressed in proximal and distal segments of the rat nephron. N-cadherin is the predominant cadherin in the proximal tubule, but is essentially absent in other nephron segments. By contrast, E-cadherin is abundant in the distal tubule, collecting duct and most medullary segments, but is present only at very low levels in the proximal tubule. In addition, the various segments of the proximal tubule exhibit different patterns of N-cadherin labeling. The S1 and S2 segments exhibit a fine threadlike pattern of labeling at the apical cell surface, whereas the S3 segments show intense labeling at the lateral cell-cell contacts. These findings raise the possibility that differences in cadherin expression and localization may contribute to the differences in the susceptibility of various nephron segments to renal pathology or nephrotoxic injury.

Methods

Animals and Processing of Tissue Samples

Male Sprague-Dawley rats weighing 250–300 grams were deeply anesthetized with a mixture of ketamine/xylazine (67/7 mg/kg IP). The abdominal cavity was opened, and the kidneys were removed and cut sagitally into 3 segments. The segments were immediately frozen in Cryomatrix (Thermo Shandon, Pittsburgh, PA) in a bath of liquid N_2 and then stored at -80°C until sectioned. The frozen samples were sagittally sectioned at a thickness of 5 μ and the sections were transferred to Superfrost Plus glass slides (Fisher Scientific, Pittsburgh, PA). The sections were then stored at -80°C until they were processed for the visualization of the molecules of interest.

Visualization of Cadherins and β-Catenin

N-Cadherin, E-cadherin and β-catenin were visualized by indirect immunofluorescence techniques. In the first series of experiments, a dual labeling procedure was used to visualize E-cadherin and N-cadherin in the same sections. Cryosections were permeabilized in -20°C methanol for 10 minutes and blocked in 3% goat serum for 15 minutes. They were then incubated for 1 hour with the primary antibodies, a mouse anti-human E-cadherin (B-D Transduction Labs #C20820, San Diego, CA) and a rabbit antihuman N-cadherin (Calbiochem #205606, LaJolla, CA). The samples were washed in phosphate buffered saline (PBS) and incubated for 40 minutes in the secondary antibodies, a FITC-conjugated goat anti-mouse IgG (Sigma #F0257, St. Louis, MO) and a TRITC-conjugated goat anti-rabbit IgG (Sigma #T5268, St. Louis, MO). The samples were then washed in deionized water, covered with glass coverslips over AquaPolymount (Polysciences, Inc., Warrington, PA) and viewed with a Nikon Eclipse 400 fluorescence microscope using either 40× or 100× objectives.

In another series of experiments, a dual-labeling procedure was used to visualize β-catenin and either E-or N-cadherin in the same sections. Samples were fixed and permeabilized for 10 minutes in -20°C methanol and blocked in 3% goat serum for 15 minutes. The samples were then incubated for 1 hour in the primary antibodies, a rabbit polyclonal anti-β-catenin (Zymed #71-2700, South San Francisco, CA) and either a mouse anti-human E-cadherin or a mouse anti-human N-cadherin (BD Transduction Labs #C70320). The samples were washed in PBS and incubated for 40 minutes in the secondary antibodies, a TRITC-conjugated goat anti-rabbit IgG and a FITC-conjugated goat anti-mouse IgG. Samples were then washed in deionized water, mounted and viewed as previously described.

In the last series of experiments, dual labeling procedures were used to visualize either E-cadherin or N-cadherin and specific markers for proximal and distal nephron segments. The specific markers that were labeled included aquaporin 1, which is normally expressed in the proximal tubule, and to a lesser extent in the descending thin limb of the loop of Henle [42,43], and aquaporin 2, which is normally expressed in the collecting duct [44,45]. Samples were fixed and permeabilized in -20°C methanol for 10 minutes and blocked in 3% horse serum for 15 minutes. The samples were then incubated for 1 hour in the primary antibodies, either a mouse anti-human N-cadherin (B-D Transduction Labs #C70320) or a mouse anti-human E-cadherin (B-D Transduction Labs #C20820) and either a rabbit anti-rat aquaporin 1 (Alpha Diagnostics International, San Antonio, TX #AQP11-5) or aquaporin 2 (Alpha Diagnostics International #AQP21-

5). Samples were rinsed in PBS and incubated for 45 minutes in the secondary antibodies, a TRITC-conjugated goat anti-mouse IgG (Sigma #T5393) and a FITC-conjugated goat anti-rabbit IgG (Sigma #F0382). Samples were then rinsed, mounted and viewed as described previously.

For most of the studies, digital images were captured with a Spot digital camera (Diagnostic Instruments, Sterling Heights, MI) using automated exposure times and gain settings for the bright-field images. The dark-field fluorescence images of E-cadherin, N-cadherin and β-catenin labeling were captured using a gain setting of 16 for FITC and 8 for TRITC, and exposure times of 3 s for green and 1 s for red and blue. The digital images were processed using the Image-Pro Plus image analysis software package (Media Cybernetics, Silver Springs, MD). To further characterize the patterns of cadherin labeling in specific nephron segments, some of the samples were also examined using an automated focusing system and deconvolution technique to obtain images of a series of optical planes through the section. For these studies, the sections were viewed with a Nikon E800 microscope fitted with a Spot RT SE6 camera (Diagnostic Instruments) and a Z-axis controller interfaced with the Image Pro Plus 4.5 software package (Media Cybernetics) on a Dell 8200 computer. A stack of 20 images was acquired in 0.3 μ increments using a 100× objective. To remove out-of-focus information from each image in the stack, the images were subjected to a blind deconvolution algorithm using AutoDeblur software (AutoQuant Imaging, Inc., Watervliet, NY) to yield a frame-by-frame reconstruction of the specimen.

Negative controls for all labeling studies consisted of kidney sections that were incubated without the primary antibodies, as well as sections that were incubated with non-diluted, non-immune serum from the same species in which the primary antibodies had been generated. To rule out the possibility that any apparent labeling may have resulted from fluorescence "spill over" when the FITC labeled samples were viewed with the TRITC filter configuration and vice versa, all experiments also included a group of samples that were labeled with each of the TRITC- or FITC-tagged antibodies individually. When the TRITC-labeled samples were viewed with the FITC filter configuration and the FITC-labeled samples were viewed with TRITC filters, no labeling was evident in any of the samples. All labeling experiments were repeated at least three times and appeared to be highly reproducible.

List of Abbreviations

FITC fluorescein isothiocyanate

TRITC tetramethylrhodamine isothiocyanate

Authors Contributions

W.C.P. designed and directed the study and did most of the photography and morphologic analyses. P.C.L. prepared all cryosections and did most of the immunofluorescence labeling. D.M.A. performed the deconvolution analyses. All authors read and approved the final manuscript.

Additional material

Additional File 1

The data file shows images of the N-cadherin labeling in a series of optical planes through a typical field of the outer cortex. These images were generated using the automated focusing system and deconvolution technique described in the Methods. The threadlike pattern of N-cadherin labeling near the apical cell surface of the cells in the S1/S2 segment of the proximal tubule can best be seen by clicking on the small black triangular cursor to the middle region of the scale shown just below the viewing screen.
Click here for MPEG version of file
[http://www.biomedcentral.com/content/supplementary/1472-6793-4-10-S1.mp4]
Click here for MOVIE version of file
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Acknowledgements

This work was supported by Grant R01 ES006478 from the National Institute of Environmental Health Sciences. The authors gratefully thank Victoria Sears for her assistance in processing the digital photos and for her help in preparing the manuscript.

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