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Angiotensin-converting enzyme in non-neoplastic kidney diseases

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Background. The angiotensin I-converting enzyme (ACE, CD143, kininase II) plays a critical role in controlling the level of vasoactive peptides such as angiotensins and kinins in the local circulations and tissue interstitium. Because recent work has documented a vessel-, organ-, and species-specific pattern of endothelial ACE expression in the vascular system, we have analyzed whether or not changes of this pattern occur in vessels, tubules, and interstitium of the human kidney that is affected by different non-neoplastic diseases.

Methods. Using a set of well-characterized monoclonal antibodies (mAbs), ACE was assessed on renal tissue of 135 patients by immunohistochemistry, including an additional analysis at the ultrastructural level. A semiquantitative evaluation allowed the estimation and comparison of ACE content in different renal compartments. These data were compared with several clinical findings, diagnosis, therapeutic modalities, and histological features.

Results. In contrast to the normal human kidney, where ACE is abundant in the brush border of the proximal tubule but is usually absent in endothelial cells of any vessel type, an endothelial neoexpression of ACE was observed in different diseases. In general, this neoexpression was associated with histological sites of interstitial fibrosis and showed some selectivity for glomerular endothelial cells in diabetes mellitus and chronic arterial hypertension. There was also a loss of epithelial ACE in the proximal tubule in certain pathological conditions, for example, in chronic fibroplastic processes, acute pyelonephritis, and different stages of acute renal failure.

Conclusions. Neoexpression of ACE by renal endothelial cells, as well as changes of the tubular ACE content, is a common finding in diseased human kidneys. As associated with certain tissue sites, clinical and/or morphological features, these changes may be involved in parenchymal remodeling and renal pathophysiology.

Angiotensin-converting enzyme (ACE, kininase II, E.C.3.4.15.1) is a zinc metallopeptidase that mainly acts as a dipeptidylpeptidase and has a broad specificity capable of hydrolyzing a wide range of peptide substrates *in vitro*. ACE plays an important role in the regulation of blood pressure and the control of regional blood flow through the generation of angiotensin II (Ang II) from angiotensin I and the degradation of bradykinin. Two isoforms of the molecule are known: a somatic and a testicular form. In contrast to testicular ACE, exclusively expressed by germinal cells, the somatic isoform of ACE is found in different cell types as a transmembrane ectoenzyme [1, 2]. Besides its vascular localization on the luminal surface of endothelial cells [3, 4], somatic ACE is present in other cell types such as neuroneal cells, absorptive epithelial cells, and mononuclear cells of the immune system [1, 2, 5, 6, 9] [abstracts: Chumachenko et al, *Arteriosclerosis* 115(Suppl):S63, 1995; Metzger et al, *Pathol Res Pract* 192:364, 1996]. Especially the kidney is well known to contain high amounts of ACE. Former studies localized the enzyme mainly to the brush border of the proximal tubular segment in humans and other species [10–14].

By newly introduced monoclonal antibodies (mAbs), the detection of immunoreactive ACE has become reliable [5, 14], and consequently, the antigen was assigned to CD143 [15, 16]. Some of these mAbs are also suitable for formalin-fixed, paraffin-embedded tissue specimens, allowing studies on human tissue archives of certain organ systems or a multitude of diseases (abstract; Metzger et al, *Pathol Res Pract* 192:364, 1996). Thus, we have recently shown that the expression of ACE within the human vascular system is heterogeneous, largely depending on the localization of the endothelial cell and the vessel type. Only endothelial cells of small muscular arteries and arterioles show a strong anti-ACE immunoreactivity in the systemic circulation combined with an organ as well as species dependent pattern (Franke et al, manuscript submitted for publication).

One of the most remarkable exceptions that has been documented during these studies concerns the kidney. In contrast to the overall systemic circulation and animal

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species [17–19], endothelial cells of the normal human renal vascularization usually completely lack ACE. To reconfirm this particularity at the ultrastructural level also, we investigated the pattern of ACE distribution in a large number of human kidney specimens. Striking changes of the normal expression pattern of ACE were observed in different diseases and non-neoplastic renal tissue alterations, both in epithelial and endothelial cells. These changes were analyzed in relationship with clinical, diagnostic, and therapeutic data of the patients, as well as disease-specific alterations of tissue morphology. Thus, this study may: (*a*) prove ACE to be neoexpressed or lost in renal pathology, (*b*) hint to particular associations of some of these changes with parenchymal remodeling, and (*c*) give a rough survey of those conditions in which changes of the renal ACE expression most likely occur.

METHODS

Patients and tissue samples

Renal tissues of 135 patients, 77 males and 58 females, were analyzed. The mean age of patients was 65.5 and ranged from 17 to 92 years. Fresh tissue samples from 79 human kidneys were obtained within minutes after surgery. The kidneys were removed for either renal cancer ($N = 64$) or for complications of acute or chronic inflammations ($N = 15$). All specimens were taken from organ parts not affected by a neoplastic process. They were directly fixed in phosphate-buffered formalin for 24 hours and were routinely paraffin embedded. Additionally, eight of these kidneys were obtained either snap frozen in liquid nitrogen for native immunohistochemistry and immunofluorescence or were fixed by immersion for four hours at 4°C with 4% paraformaldehyde in phosphate-buffered saline (PBS) for ultrastructural immunocytochemistry. In addition, renal tissues of 56 deceased patients were collected on the occasion of an autopsy within 24 hours after death of quite different causes. The tissue specimens were either snap frozen in liquid nitrogen or were formalin fixed and paraffin embedded as described. For the demonstration of species-specific differences, kidney specimens of eight rats (4 of Wistar strain and 4 of Lewis strain) were taken fresh and snap frozen in liquid nitrogen.

Histological diagnosis

The morphologic evaluation focused on the following diagnoses: arteriosclerosis, arteriolosclerosis, cortical fibrosis, medullar fibrosis, glomerulosclerosis, loss of glomeruli, acute pyelonephritis, chronic interstitial inflammation, and signs of shock, as defined by tubular dilation and protein content, epithelial dissociation, tubular necrosis, and vascular microthrombosis. Each of these pathological features was divided into four grades (0, 1, 2, and 3) characterizing the progress of tissue alteration. For example, arteriosclerosis was graded as follows: 0 = normal artery; 1 = beginning of intimal thickening with a reduction of the arterial lumen of no more than 20%; 2 = intimal thickening with a reduction of the arterial lumen between 21 to 80% and beginning of structural disorder of the media; and 3 = stenotic artery with a reduction of the arterial lumen of more than 81% and complete structural disorder of the media. The grades were independently determined by three morphologists (R.M., R.M.B., and F.E.F.) on hematoxylin and eosin (H&E) and on periodic acid-Schiff reaction (PAS)-stained tissue sections. Histological grading of tissue alterations differed by no more than one point between the observers. Concerning only those morphological features of advanced lesions (grades 2 and 3) and those cases that were definitely analyzable, the distribution was as follows: arteriosclerosis, 91 of 135; arteriolosclerosis, 35 of 135; cortical fibrosis, 27 of 135; medullar fibrosis, 32 of 116; glomerulosclerosis, 19 of 135; loss of glomeruli, 21 of 135; acute pyelonephritis, 3 of 135; chronic interstitial inflammation, 25 of 135; and signs of shock, 19 of 132.

Clinical diagnosis

For the majority of patients, additional clinical data were available and were obtained from the medical and clinical records of the Departments of Internal Medicine, Urology, and Surgery of the Justus-Liebig-University of Giessen. Besides the recorded and proven presence or absence of the clinical diagnoses of chronic arterial hypertension ($N = 32$ of 119), diabetes mellitus ($N = 12$ of 114), chronic heart failure ($N = 30$ of 114), and renal insufficiency ($N = 14$ of 114), in at least 99 cases they included additional information about the body size and weight of patients, mean blood pressure values during the time of hospitalization, laboratory findings (serum levels of sodium, potassium, urea, creatinine), as well as information about therapeutically administered drugs such as ACE inhibitors ($N = 10$ of 99), β-blockers ($N = 7$ of 99) and corticosteroids ($N = 6$ of 99).

Slide preparation and pretreatment

Native tissues were sectioned at 5 μm by a cryostat microtome (CM 3000; Leica/Jung, Heidelberg, Germany). The slides were air dried at room temperature (RT) for 12 to 24 hours, and then they were either processed directly or stored at −30°C. Formalin-fixed, paraffin-embedded tissues were sectioned at 2 to 4 μm (SM 2000 R; Leica/Jung) followed by an overnight drying at 37°C in an incubator. Before immunohistochemical staining, the paraffin sections were dewaxed in xylene for 10 minutes, followed by 10 minutes of acetone and 10 minutes of acetone/Tris-buffered saline (TBS; 1:1). After this treatment, the slides were washed in TBS,

Table 1. Antibodies to human angiotensin-converting enzyme (ACE; CD143) and endothelial cells

Clone/serum	Immunogen	Concentration μg/ml	Source/references
9B9	Human lung ACE (CD143)	0.4	Chemicon, CA [20]
3G8	Human lung ACE (CD143)	5.0	Chemicon, CA [20]
i1A8	Human lung ACE (CD143)	33.0	Chemicon, CA [20]
i2H5	Human lung ACE (CD143)	1.0	Chemicon, CA [20]
3A5	Human lung ACE (CD143)	6.7	Chemicon, CA [20]
5F1	Human lung ACE (CD143)	10.0	Chemicon, CA [20]
CG1[a]	Human kidney ACE (CD143)	10.0	[20]
CG2[a]	Human kidney ACE (CD143)	5.0	BMA, Augst [20]
CG4[a]	Human kidney ACE (CD143)	10.0	[20]
Y4[b]	Human kidney ACE (CD143)	2.0	[13]
JC/70A	CD31 (PECAM-I)	2.3	DAKO, Glostrup
10F3	CD54 (ICAM-I)	1.0	GLT Biotrack, NY
QBEND/10	CD34	2.0	Dianova, Hamburg
F8/86	vWF	11.6	DAKO, Glostrup
BMA 120	Endothelium	10.0	Behring, Marburg
BNH 9	BUR cell line	Pre-diluted	Dianova, Hamburg
MRC OX-43	Rat endothelium	1:50,000	Serotec, Wiesbaden
MR12/53[c]	Rabbit Ig	1.0	DAKO, Glostrup

[a] Reactive on microwave pretreated formalin fixed tissue sections
[b] Purified immunoglobulin fraction from rabbit antiserum
[c] Used as negative control in APAAP technique

placed in microwave-proofed tubes (Sigma, Deisenhofen, Germany) containing 0.01 mol citrate buffer solution (pH 7.0) and were boiled for five minutes at 600 W in the microwave (SS 566H; Bosch, Munich, Germany). The evaporated volume was replaced with distilled water, and the procedure was repeated four times. After microwave treatment, the slides were left to cool down and were washed in TBS. Frozen sections were fixed in acetone for 10 minutes at RT and were air dried.

Antibodies

Nine well-characterized mouse mAbs to the somatic isoform of human ACE (CD143) were used [20]. One set of these mAb (9B9, i2H5, 5F1, 3G8, 1A8, and 3A5) has been generated to the native, conformational intact ACE molecule of the lung. The other set (CG1, CG2, and CG4) generated to human kidney ACE recognizes the slightly denatured molecule. In addition, one highly purified polyclonal antibody (pAb) to human kidney ACE made in rabbit, Y4, was used [13]. To ensure endothelial cell integrity, mAbs known as endothelial cell-specific markers were applied: CD31, CD34, CD54, factor 8, BMA 120, and BNH 9. A mouse antirabbit-Ig mAb (MR 12/53) was used as negative control (Table 1). For the detection of ACE in the kidney of rat, mAb 9B9, cross reacting with ACE of rat on native tissues [5], was used. Here, the reactivity was compared with an endothelial cell marker of rat (mAb MRC OX-43) [21]. The proper concentration of each mAb was thoroughly tested for the optimal staining signal on native and formalin-fixed tissues as recently described in detail (Franke et al, manuscript submitted for publication). The final concentrations used are given in Table 1.

Immunohistochemistry

The alkaline-phosphatase-antialkaline-phosphatase (APAAP) technique was applied according to the method of Cordell et al [22]. On native and formalin-fixed tissue sections, the technique was applied with the following modifications: To prevent nonspecific reactivity [23], the polyclonal rabbit antimouse Ig ("link"-antibody; Dako, Glostrup, Denmark) was used at a dilution of 1:40. When staining native tissues of rat, the polyclonal rabbit antimouse Ig was preabsorbed by an admixture of purified rat serum. Here, a 1:750 dilution of redistilled lyophilized rat serum (Dianova, Heidelberg, Germany) was found to be optimal to abolish any background. The APAAP complex (Dako) was used at a dilution of 1:50. The alkaline phosphatase substrate reaction with new fuchsin (100 μg/ml) and levamisole (400 μg/ml) was performed for 20 minutes at RT. Additionally, other immunohistochemical methods such as the silver-amplified immunogold technique [24] and conventional immunofluorescence were performed to confirm the results of immunostaining allowing double-labeling experiments.

Ultrastructural immunocytochemistry

To analyze ACE in renal tissues at the subcellular level, the pre-embedding method combined with the peroxidase-antiperoxidase (PAP) staining method was applied [25]. Fixed human kidneys, cryoprotected in 2.3 molar sucrose in PBS, were snap frozen in liquid nitrogen and sectioned into 25 to 30 μm thick slices by a cryostat

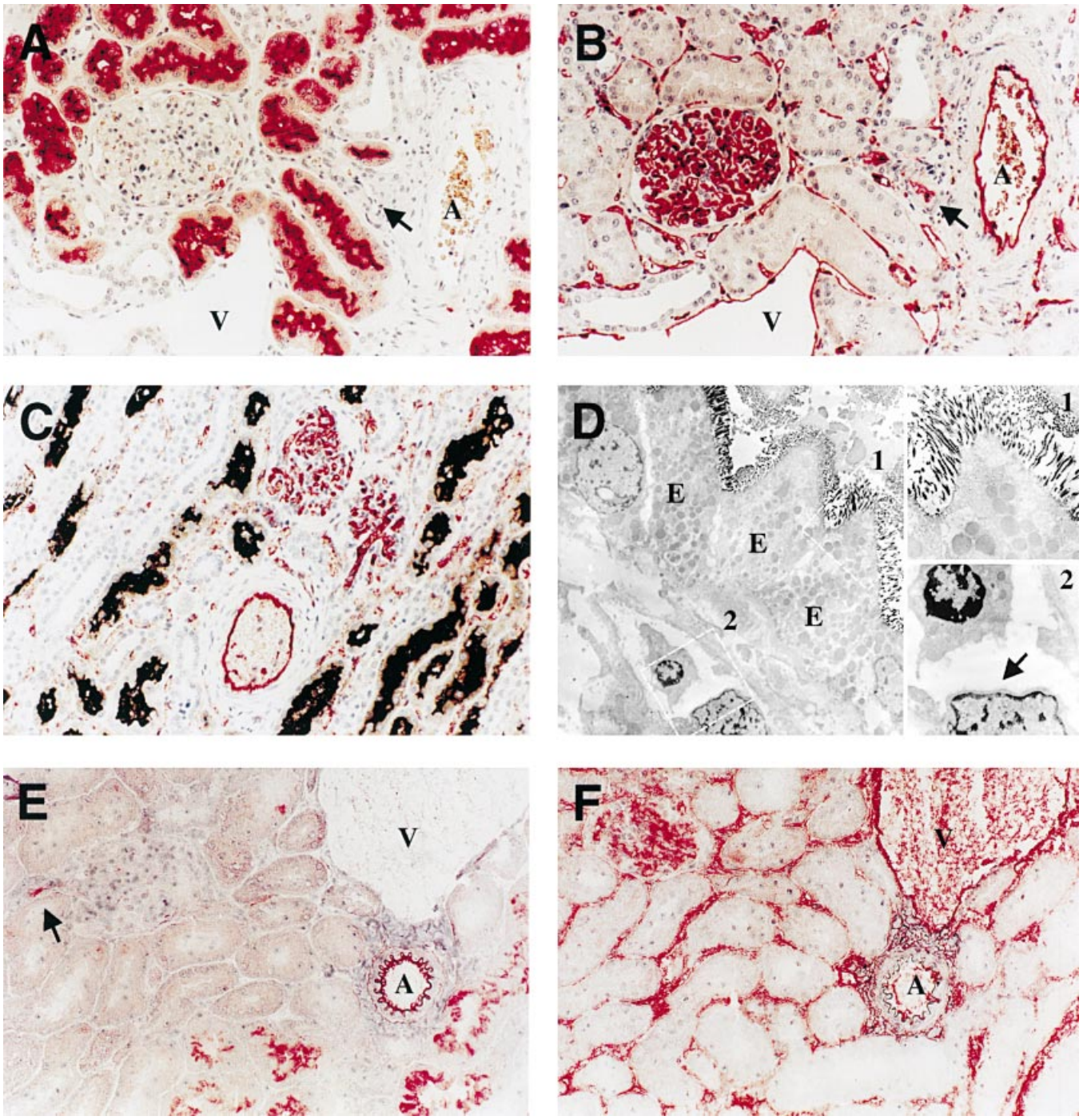

Fig. 1. Angiotensin-converting enzyme (ACE; CD143) in the normal kidney of humans (*A–D*) and of rat (*E* and *F*). (*A*) The typical apical cellular distribution of ACE is shown on epithelial cells of the proximal tubule, whereas those of the distal tubule are negative. Also not labeled for ACE are all endothelial cells, as shown by an interlobular artery (A), a vas afferens (arrow), a glomerulus, and a vein (V). Compare with the serial section and endothelial marker CD31 at the right side (anti-ACE mAb CG1, APAAP, paraffin-section ×40). (*B*) A serial section of the same topography is showing all endothelial cells homogeneously stained by CD31. Compare with ACE expression sites on the left by the help of the same indicated morphological structures (anti-CD31 mAb JC/70A, APAAP, paraffin-section ×40). (*C*) Double labeling to ACE (CG2, black) and to CD31 (JC/70A, red) at the border of the renal cortex and the medullary ray. Endothelial ACE immunoreactivity is not detectable (anti-ACE mAb CG2 and anti-CD31 mAb JC/70A, silver-amplified immunogold and APAAP, paraffin-section ×40). (*D*) Ultrastructural detection of ACE is limited exclusively to the apical brush borders of the proximal tubular epithelial cells (*E*). No protein precursors in cytoplasmic vesicles and no ACE on basolateral cell membranes are visible. Insert 1 shows a magnification of the upper part of a single epithelial cell with the heavily marked brush-border. Insert 2 shows a magnification of the surface of a capillary endothelial cell (arrow) lacking ACE, as well as a plasma cell in the lumen of the capillary (anti-ACE mAb CG2, pre-embedding method with PAP, X 600/insert 1 and 2 × 1200). (*E*) The epithelial ACE distribution in the kidney of rat shows a moderate expression only at the distal part of the proximal tubule. Also, endothelial ACE is clearly present in an interlobular artery (A) and a vas afferens (arrow), whereas endothelial cells of the glomerule, the capillary network, and a vein (V) are not labeled. Compare with serial section and endothelial marker MRC OX-43 on the right hand and with the different expression pattern of humans above (anti-ACE mAb 9B9, APAAP, cryostat-section, ×40). (*F*) Serial section of the same topography is showing all endothelial cells stained by MRC OX-43. Note that this mAb also cross-reacts with stromal cells and blood ingredients. Compare with ACE expression sites on the left by the help of the same indicated morphological structures (mAb MRC OX-43, APAAP, cryostat-section, ×40).

microtome. After descending and ascending alcohol steps in PBS, the slices were incubated for one hour at RT with a blocking solution of 10% normal goat serum/PBS (Sigma, St. Louis, MO, USA). The slices were then incubated with the primary mAb CG2 at a dilution of 20 μg/ml in PBS for one hour. This was followed by incubation with the polyclonal rabbit antimouse Ig, used at a dilution of 1:20 in PBS and finally by incubation with mouse PAP (Dako) used at a dilution of 1:80 in PBS. Each incubation step was done for one hour at RT and was followed by washing three times for 10 minutes. The sections were then rinsed for 20 minutes in 0.05 M Tris-HCl buffer, pH 7.6, and the peroxidase activity was revealed by incubation with 10 mg diaminobenzidine (DAB; Sigma) in 10 ml 0.05 M Tris-HCl buffer, pH 7.6, containing 5 μl of H_2O_2 (30%). After fixation and DAB amplification in 1.25% osmium tetroxide [26], the slices were dehydrated and flat embedded in standard Spurr. Contrasted thin sections were examined in a Zeiss EM 9 S-2 electron microscope at 60 kV.

Semiquantitative evaluation and statistics

The renal expression of ACE was evaluated regarding different anatomic structures as mentioned earlier in this article. The tubular compartment was analyzed in 135 cases by immunohistochemistry; the complete renal vascularization, including all parenchymal vessel types, was assessed in 106 of these cases. The levels of immunoreactivity in different renal cells and compartments were scored as previously described [27, 28] by three morphologists (discussed earlier in this article) into four subgroups as follows: 0 = no expression detectable; 1 = slight expression; 2 = moderate expression; and 3 = strong expression. For example, the score value of 3 was equivalent to the immunostaining of proximal tubule epithelial cells by mAb CG1 (Fig. 1A) or immunostaining of endothelial cells by the mAb to CD31 (PECAM-I; Fig. 1B). Again, the scoring differed not essentially between the three observers. The statistical evaluations were performed using a computer program [SPSS™ for Windows 95™ (release 8)]. Clinical and morphological diagnoses as well as patients data were correlated with the level of ACE (score values) in anatomic structures using the Spearman rank correlation method, which is appropriate for ordered categorical data. The Spearman rank correlation coefficient [$r_{(s)}$, possible values from -1 to 1] measures the degree of monotone associations between two variables: A large positive value of $r_{(s)}$ indicates that large (small) values of one of the ordinal variables tends to be associated with large (small) values of the other. A large negative value of $r_{(s)}$ indicates just the opposite scenario. This method gave information about the strength and direction of associations between the variables. It was combined with Pearson's chi-square test for independence and, in the case of small subgroups, with Fisher's exact test. This procedure was used to gain hints of possible relationships of the analyzed variables. Furthermore, the same method was used to inform of possible dependencies on other variables by cross-correlation, splitting of the test collective according to the factors of interest, or by the exclusion of special cases, for example, patients suffering from sepsis prior to death.

Effect of postmortem autolysis on renal angiotensin-converting enzyme immunoreactivity

To determine the influence of autolysis on the renal morphology as well as on the immunoreactivity of anti-ACE mAbs, freshly obtained renal tissue specimens of five individuals were placed in a refrigerator at 4°C in a humid atmosphere (the storing temperature of deceased patients) or in an incubator at 37°C. At certain time intervals, parts of the specimens were taken and were immediately prepared for immunohistochemical analysis.

RESULTS

Angiotensin-converting enzyme and the effect of postmortem alterations

At saturating concentrations, all anti-ACE mAbs showed an identical pattern of staining in the different human organs tested, including kidney. No differences were found between antibodies predominantly reacting on native tissues (mAb 9B9, i2H5, 5F1, 3G8, 1A8, 3A5, pAb Y4) and those predominantly reacting on formalin-fixed tissues (CG1, CG2, and CG4) [16]. ACE in the human kidney was found to be very stable with all mAbs tested (mAb 9B9, i2H5, 3G8, CG1, CG2, and CG4). Even after four days of observation, the tissues stored at 4°C showed no alterations of the intensity of immunostaining or in the pattern of distribution. Progressive autolysis occurred in renal tissues stored at 37°C. Here, even if disintegration of the renal morphology occurred, immunoreactivity was not diminished during the first 12 hours (not shown). The decrease in the intensity of immunostaining with anti-ACE mAbs occurred in a proportional manner, both for cells expressing high levels of ACE (for example, proximal tubular epithelial cells) and cells expressing low or intermediate levels (for example, endothelial cells in perirenal tissue; data not shown). We and other authors have recently described the strong molecular resistance of ACE to autolysis in human tissues [16, 29]. These data show that immunohistochemical analysis of ACE is possible on routine autopsy specimens and not only on specimens obtained from rapid autopsy or surgery.

Angiotensin-converting enzyme in normal human kidney

In all analyzed cases with a normal appearance of the renal parenchyma, a dense immunostaining with all anti-

ACE mAbs lined the luminal surface of the proximal tubule. Some epithelial cells of the parietal layer of the Bowman's capsule at the urinary pole were accessory labeled. Other segments and structures of the nephron were found to be negative (Fig. 1 A–D). No endothelial ACE was detectable in the vasculature of the human kidney. ACE was not detectable in medium- or small-sized arteries, in arterioles, in the glomerular tuft, or on the venous side of the renal parenchymal vasculature (Fig. 1 A–D). This lack of ACE was still evident when mAb concentrations were disproportionately increased, causing a strong "background" reactivity (data not shown). These findings in the renal vasculature contrast to those made in other peripheral circulations where endothelial cells were predominantly labeled by anti-ACE mAbs in small arteries and arterioles and some capillaries (Franke et al, manuscript submitted for publication). The typical expression pattern of the peripheral microcirculation occurred immediately outside the parenchymal vasculature in the loose connective tissues surrounding the calyces and the capsule (data not shown). The electronmicroscopical analysis confirmed the microscopical findings. Ultrastructurally, the labeling was confined to the cell membrane of the brush border in the epithelial cells of proximal tubule. Structures within the cytoplasm and the basolateral wall of the epithelial cells as well as the endothelial cells of the vasculature were not labeled (Fig. 1D).

Angiotensin-converting enzyme in normal rat kidney

In comparison and in contrast to the human in which intense labeling was observed all along the proximal tubule, epithelial ACE in the rat was restricted to the straight portion of the proximal tubule located at the outer zone of the medulla (Fig. 1E). By opposition to the endothelial cells of the human renal vasculature, rat kidney vessels showed a strong ACE immunoreactivity in endothelial cells of renal arteries and preglomerular arterial vessels, including the vas afferens (Fig. 1 E, F). Endothelial cells of the glomerular tuft, the capillary network, and the veins were mostly negative (Fig. 1 E, F).

Angiotensin-converting enzyme in human kidney diseases

In contrast to the normal human kidney, the pattern of ACE distribution in different renal disorders was altered. In general, a neoexpression of endothelial ACE within the vascular tree of kidney (Fig. 2 A–C) and a reduction or rearrangement of epithelial ACE in the proximal tubule were observed (Fig. 2 D–F and Fig. 3 A–F). These changes of ACE expression were scored in up to 135 adult kidneys at defined tissue sites and were correlated with each of several clinical findings, diagnosis, and histological tissue alterations. The most important results are summarized in Tables 2 and 3.

Changes of tubular angiotensin-converting enzyme (CD143). A gross reduction of detectable tubular ACE was caused by any loss of intact renal proximal tubules and ACE-bearing epithelial cells. This was especially true for advanced lesions of parenchymal remodeling, and, combined with the loss of glomerules, cortical and/or medullar fibrosis (Fig. 2 A, B), raised laboratory values of retention as well as renal insufficiency (data not shown). Advanced lesions of parenchymal remodeling, including most cases of chronic pyelonephritis, also more often showed a reduced expression of ACE at the brush border sites of remaining epithelial cells (Fig. 2D and Table 2). A strictly different pattern was found in three cases of acute pyelonephritis (Fig. 2E). Unrelated to other analyzed variables, a significant loss of ACE at the epithelial brush border site and even a relative diminished detection of released tubular ACE were recorded (Table 2). This tubular reduction of detectable ACE in acute inflammation histologically coincided with the local presence of neutrophilic granulocytes, and a residual expression of ACE was sometimes only observed in additionally invaded macrophages (Fig. 2F).

Moreover, by analyzing different stages of renal shock, we found an increasing content of released and non–cell-bound ACE in the lumen of renal tubules (Fig. 3). The immunoreactivity of all anti-ACE mAbs was not diminished and was found in the form of intratubular precipitates and on the surface of cytoplasmatic blebs released by the apical membranes of the epithelial cells even in early proximal tubular injury (Fig. 3 A–D). In advanced stages of tubular damage and necrosis with loss of cellular polarity, integrity of tight junctions and cytoskeletal networks in proximal tubular cells, ACE appeared on the whole cellular surface (Fig. 3 E, F). This contrasted sharply with the polarized apical immunostaining in normal tubules (Fig. 1 A, C). The immunohistological finding of a probably intact molecule within the tubular lumen correlated well with the histological signs of renal shock (Table 2). In only cases of chronic arterial hypertension and administered corticosteroids [$r_{(S)} = 0.239$, $P = 0.017$, $N = 99$] was a relative increase of tubular released ACE also recorded. Interestingly, both were found to be not influenced by other variables, including the morphological signs of shock (in applying cross-correlation and/or exclusion of cases from analysis, for example, with signs of shock). Further clinical and therapeutical data were not associated with significant changes of tubular ACE.

Changes of endothelial angiotensin-converting enzyme (CD143). In different diseases, an unexpected and often only scattered and inhomogeneous neoexpression of ACE by endothelial cells was observed in almost all parts of the renal vascularization. Sometimes apparent even in medium- and small-sized parenchymal arteries (Fig. 2A), this neoexpression was found to more often

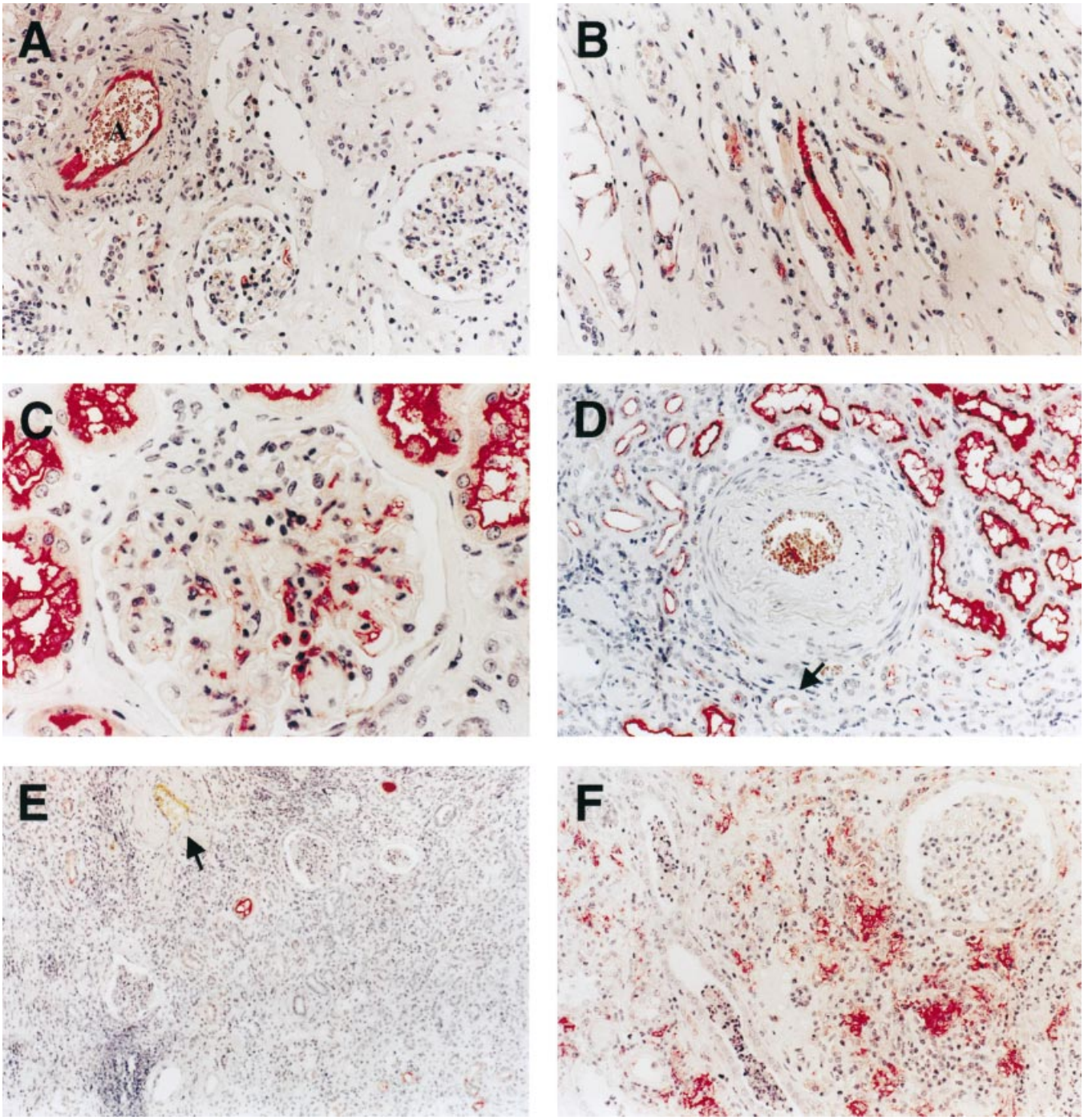

Fig. 2. Human kidney diseases with neoexpression of endothelial angiotensin-converting enzyme (ACE; CD143; *A–C*) and reduction or loss of epithelial ACE (*D–F*). (*A*) Cortical fibrosis in a case of a chronic interstitial nephritis is going along with a remarkable neoexpression of ACE in endothelial cells of a parenchymal artery (A). Endothelial cells of some glomeruli also show scattered ACE (anti-ACE mAb CG1, APAAP, paraffin-section, ×40). (*B*) Medullar fibrosis in another case of chronic interstitial nephritis shows endothelial neoexpression of ACE in vasa recta (anti-ACE mAb CG2, APAAP, paraffin-section, ×40). (*C*) Diabetic glomerulosclerosis shows neoexpression of endothelial ACE especially within the glomerular tuft. Note the diffuse enlargement of the capillary loops and the beginning of nodular capillary obliteration (anti-ACE mAb CG2, APAAP, paraffin-section, ×160). (*D*) Loss and reduction of epithelial ACE (arrow) is shown in a zone of atrophic tubules in a case of advanced arteriosclerosis. Note the intimal thickening of the parenchymal artery in the center (anti-ACE mAb CG4, APAAP, paraffin-section, ×40). (*E*) Almost complete loss and down regulation of tubular ACE in a case of acute pyelonephritis. In the acute phase of inflammation, no endothelial neoexpression is observed (arrow). Compare with the intense tubular ACE expression of normal cortical tissue (Fig 1A; anti-ACE mAb CG2, APAAP, paraffin-section, ×25). (*F*) Despite the massive loss of tubular ACE in acute pyelonephritis, invading activated macrophages may show an expression. Note that the tubular loss of ACE coincides with the presence of granulocytes (anti-ACE mAb CG2, APAAP, paraffin-section, ×40).

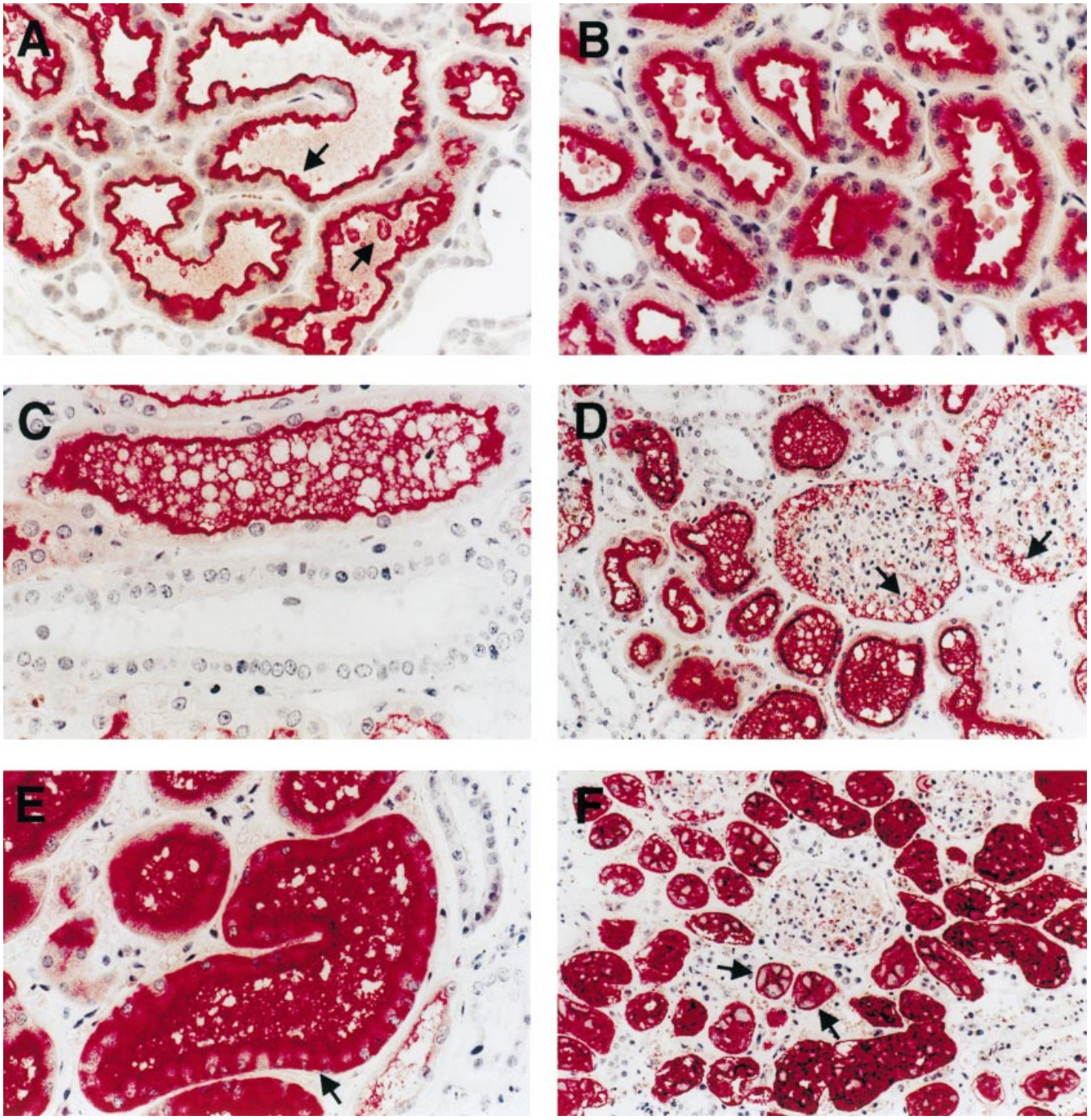

Fig. 3. Epithelial angiotensin-converting enzyme (ACE; CD143) in early (*A–D*) and advanced (*E* and *F*) stages of renal shock in humans. (A) Onset of tubular insufficiency with tubular enlargement. ACE-immunoreactive luminal precipitates and immunoreactive cytoplasmatic blebs released by the epithelial cells of proximal tubules are found (arrows; anti-ACE mAb CG2, APAAP, paraffin-section, ×160). (B) Free ACE-positive cytoplasmatic blebs and fragments are more prominent in this case of early shock stage. Note that the general tissue morphology is intact and compare with normal appearance (Fig. 1A; anti-ACE mAb CG2, APAAP, paraffin-section, ×160). (C) More intense ACE-containing precipitates and swollen blebs are shown in this early stage of shock kidney. The cellular integrity of proximal tubular epithelium is still preserved. Note the strict ACE-negative epithelium of the distal tubule (anti-ACE mAb CG2, APAAP, paraffin-section, ×160). (D) In this stage of early kidney shock, the space of Bowman's capsule is filled with ACE-reactive cellular detritus (arrows), presumably caused by flow black (anti-ACE mAb CG2, APAAP, paraffin-section, ×40). (E) The cellular ACE expression site changes with the onset of cellular dissociation in this more advanced stage of renal shock [now see the pericellular and cytoplasmatic distribution of immunoreactivity (arrow) in contrast to the normal apical expression site; Fig. 1A and discussed earlier in this article; anti-ACE mAb CG2, APAAP, paraffin-section, ×160]. (F) The cellular detachment from basal membranes, the complete loss of polarity, and the beginning of necrosis are conjoined with a circular cellular distribution of ACE (arrows). Note that even in this advanced stage of shock, the immunoreactivity to ACE obviously is not diminished (anti-ACE mAb CG2, APAAP, paraffin-section, ×40).

Table 2. Changes of tubular angiotensin-converting enzyme (ACE; CD143) in correlation to clinical diagnosis and renal morphology

	Localization of anti-ACE immunoreactivity	
	Brush borders (apical pole of epithelium)	Lumen of tubules (released ACE)
Clinical diagnosis		
Arterial hypertension	NS	$r_{(s)} = 0.187^{be}$
Diabetes mellitus	NS	NS
Cardiac insufficiency	NS	NS
Chronic renal insufficiency	NS	NS
Vascular alterations		
Arteriosclerosis	NS	NS
Arteriolosclerosis	NS	NS
Glomerular sclerosis	NS	NS
Parenchymal remodeling		
Loss of glomerules	$r_{(s)} = -0.214^{b}$	NS
Cortical fibrosis	$r_{(s)} = -0.177^{b}$	NS
Medullar fibrosis	$r_{(s)} = -0.205^{b}$	NS
Inflammatory changes		
Acute pyelonephritis	$r_{(s)} = -0.337^{de}$	$r_{(s)} = -0.211^{be}$
Chronic pyelonephritis	$r_{(s)} = -0.260^{c}$	NS
Signs of shock[a]	NS	$r_{(s)} = 0.453^{de}$

The analysis of those variables presented in rows and columns was performed according to Spearman's rank correlation test. $r_{(s)}$ = Spearman's rank correlation coefficient (possible values from −1 to 1). Abbreviation NS is no significant correlation.

[a] As defined by tubular dilation, tubular protein, epithelial dissociation, necrosis, and microthrombosis

[b] $P < 0.05$, [c] $P < 0.01$, [d] $P < 0.001$, depending on the availability of data sets of each individual case, all correlations are based on the minimum of $N = 114$ and the maximum of $N = 135$

[e] Correlations that were found not essentially affected by other variables (see text)

affect smaller vessels such as arterioles, vasa afferentia and efferentia, glomerular capillaries, vasa recta, and also the endothelial cells of veins (Fig. 2B). The endothelial neoexpression of ACE was not specific for certain tissue sites in most cases, but was observed in vessels next to fibrotic remodeling of kidney parenchyma and occurred, for example, more frequently in endothelial cells of vasa recta in the case of medullary fibrosis (Table 3).

Although found to be not specific for a certain type of disease, the glomerular neoexpression of ACE correlated with the incidence of diabetes mellitus (Fig. 2C and Table 3). In a total of 86 patients analyzable for both features, for dichotomized data this means that ACE expression was immunohistochemically detected in glomeruli of 6 out of 11 kidneys of diabetic patients but only in those of 4 out of 75 kidneys of nondiabetic patients. Interestingly, moreover, a slight glomerular neoexpression of ACE was also found to be associated more frequently with patients having the recorded diagnosis of chronic arterial hypertension (Table 3). For 91 analyzable patients again this means that glomerular ACE was present in 7 out of 27 hypertonic patients versus only 3 out of 64 nonhypertonic patients. Despite a partial coincidence of both diseases in patients suffering from diabetes mellitus and chronic arterial hypertension, the presented correlations were not found to neutralize one another or were found to be influenced by advanced morphological alterations of the glomerule (in applying cross-correlation and/or exclusion of cases from analysis). However, of those clinical and therapeutical data that were additionally compared, only the administration of ACE inhibitors in the course of the clinical presentation of the affected hypertonic patients was found significantly associated with the detection of endothelial ACE and, here, only at the site of glomerules [$r_{(s)} = 0.314$, $P = 0.008$, $N = 71$].

DISCUSSION

The abundant expression of ACE in the human kidney tubule observed in this study is essentially in agreement with previous studies [10–14]. ACE, as proven by ultrastructural analysis, was found to be strictly confined to the epithelial brush borders of the proximal tubule. We could not confirm an additional expression on the basolateral cell membrane, the endoplasmatic reticulum, or the nuclear envelope, as reported by other authors [10, 12, 13, 30]. This may be due to the use of mAb and different tissue-fixation procedures. Despite the abundance of the enzyme on tubular brush borders, its local and systemic function is not clearly understood. ACE, as a peptidase with a broad substrate specificity, is able to hydrolyze a wide range of peptide substrates *in vitro*, liberating different dipeptides or tripeptides [2]. Ang II is most likely one of the products of tubular ACE. Found in high local concentrations [31], Ang II was shown to be formed in the proximal tubular lumen [32] and was shown to stimulate the sodium and bicarbonate transport [33, 34]. Ang II receptors also have been identified in the renal brush border and basolateral cell membranes [35, 36]. Recently, concerning possible resorptive functions of ACE in addition, two high-affinity oligopeptide transporters were identified in the brush borders of intestine and proximal tubules of kidney: PepT1 and PepT2, respectively [37–41]. Because of similar cellular and subcellular localization, ACE and other peptidases might also participate in the terminal digestion and reabsorption of proteins and peptides in the tubule lumen, together with the dipeptide and tripeptide transporter PepT2.

In different diseases associated with an acute damage to the proximal tubular epithelium, we found a luminal release of ACE. Especially in advanced stages of renal shock, this non–cell-bound immunoreactivity was found to diffuse to proximal and distal segments of the nephron (Fig. 3 A–D). It has been proposed that urinary ACE and ACE activity may be a marker of proximal tubule damage [42, 43]. Our data are in agreement with these clinical and experimental findings and confirm that the urinary enzyme is released from the proximal tubular

Table 3. Changes of endothelial angiotensin-converting enzyme (ACE; CD143) in correlation to clinical diagnosis and renal morphology

	Localization of anti-ACE immunoreactivity		
	Arterioles[a]	Glomerules	Vasa recta
Clinical diagnosis			
Arterial hypertension	NS	$r_{(s)} = 0.310^{df}$	NS
Diabetes mellitus	NS	$r_{(s)} = 0.513^{ef}$	$r_{(s)} = 0.241^{c}$
Cardiac insufficiency	NS	NS	NS
Chronic renal insufficiency	NS	$r_{(s)} = 0.252^{c}$	NS
Vascular alterations			
Arteriosclerosis	NS	NS	NS
Arteriolosclerosis	NS	$r_{(s)} = 0.217^{c}$	NS
Glomerular sclerosis	$r_{(s)} = 0.272^{d}$	$r_{(s)} = 0.248^{c}$	$r_{(s)} = 0.317^{d}$
Parenchymal remodeling			
Loss of glomerules	$r_{(s)} = 0.270^{d}$	NS	$r_{(s)} = 0.304^{d}$
Cortical fibrosis	$r_{(s)} = 0.271^{d}$	NS	$r_{(s)} = 0.316^{d}$
Medullar fibrosis	$r_{(s)} = 0.258^{c}$	$r_{(s)} = 0.285^{d}$	$r_{(s)} = 0.485^{ef}$
Inflammatory changes			
Acute pyelonephritis	NS	NS	NS
Chronic pyelonephritis	$r_{(s)} = 0.274^{d}$	NS	$r_{(s)} = 0.230^{e}$
Signs of shock[b]	NS	NS	NS

The analysis of those variables presented in rows and columns was performed according to Spearman's rank correlation test; $r_{(s)}$ = Spearman's rank correlation coefficient (possible values from −1 to 1); abbreviation NS is no significant correlation.

[a] Vasa afferentia and efferentia

[b] As defined by tubular dilation, tubular protein, epithelial dissociation, necrosis, and microthrombosis

[c] $P < 0.05$; [d] $P < 0.01$; [e] $P < 0.001$; depending on the availability of data sets of each individual case, all correlations are based on the minimum of $N = 86$ and the maximum of $N = 106$.

[f] Correlations that were found not essentially affected by other variables (see text)

epithelium [44]. The loss of all brush border enzymes during ischemia occurs in general. In this respect, however, the strong molecular resistance of ACE even in advanced cellular damage and necrosis is remarkable (Fig. 3F). Only in cases of chronic arterial hypertension or therapy with corticosteroids was a tendency to a slight increase of luminal ACE also recorded. Sharply contrasting with this cellular detachment of still conserved ACE, a marked reduction or even complete loss of any detectable ACE in renal tubules occurred in acute pyelonephritis (Fig. 2 E, F). This loss of immunoreactivity in the absence of cellular necrosis may be best explained by a drastic conformational change or even molecular destruction of ACE in the close neighborhood of activated neutrophilic granulocytes. Liberated peroxides in the granulocytic burst reaction have clearly been shown to decrease ACE activity of endothelial cells in animal models [45]. Interestingly, this granulocytic burst and peroxide-induced decrease of ACE activity may indeed be paralleled by the loss of immunohistochemically detectable ACE in a cell culture model, and the reduction or loss of detectable endothelial ACE was found also in acute inflammations of other organ systems (Franke, unpublished data).

The complete lack of endothelial ACE in the normal human kidney was already mentioned in some former studies using polyclonal antibodies [10, 11]. However, in some studies, the enzyme was detected in the interstitial capillaries of the vasa recta [12–14]. We have found ACE in these particular endothelial cells in only certain pathological conditions such as medullar or cortical fibrosis. Thus, the remarkable absence of ACE in vessels of the normal human kidney in contrast to other circulations supports the concept of the heterogeneity of endothelial cell populations with tissue- and organ-specific functions [46–48]. This organ-specific lack of endothelial ACE may contribute to a relatively lower vascular resistance of human kidney and the high renal blood flow. Furthermore, the absence of endothelial ACE may protect the renal circulation against excess Ang II formation and kinin inactivation. In this respect, however, species-specific differences have to be considered, as illustrated by the different localization of renal ACE in humans and in rats (discussed in scheme of Fig. 4).

Interestingly, we found a neoexpression of ACE on endothelial cells in different diseases of the human kidney affecting certain parts of the renal vascular system, for example, arteries, capillaries of the glomerular tuft, or vasa recta. Inconstantly and independent from the nature of the underlying diseases, these changes were always seen beside local fibrotic remodeling of the affected tissue compartments. In general, a neoexpression of ACE occurs also in other cell types and diseases, for example, in sarcoidosis, atherosclerosis, myocardial infarction, and renal cell carcinoma and indicates a potential function of ACE in the angiogenesis and fibrotic parenchymal remodeling [5, 9, 16, 49, 50] [abstracts: Chumachenko et al, *Arteriosclerosis* 115(Suppl):S63, 1995; Metzger et al, *Pathol Res Pract* 192:364, 1996]. Besides vasoconstrictory effects, Ang II has potent fibrogenic and mitogenic effects [51–53], and it has been shown that the inhibition of ACE may prevent these effects in athero-

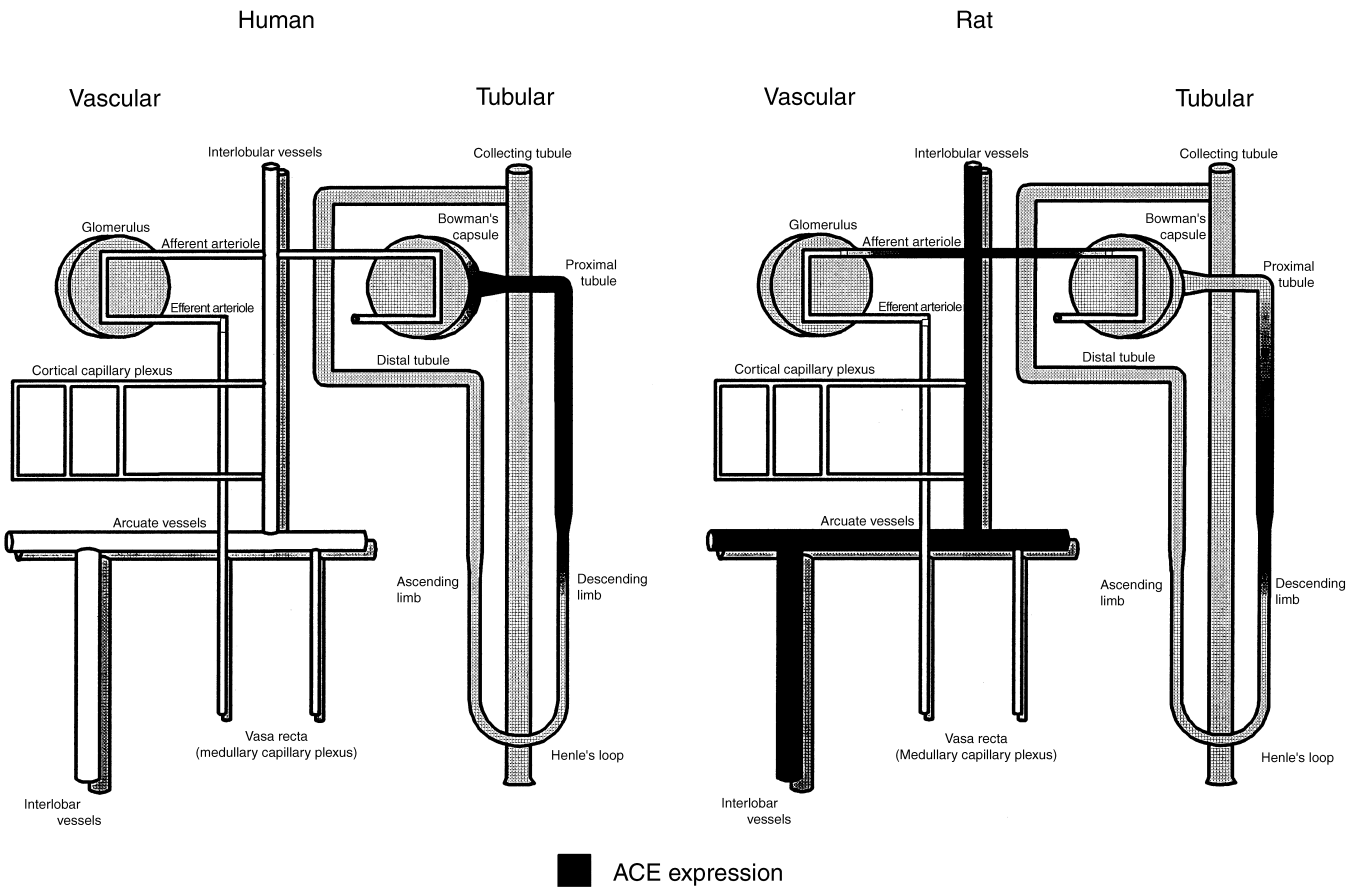

Fig. 4. Schematic diagram of the normal renal angiotensin-converting enzyme (ACE; CD143) distribution in humans and rat regarding the special endothelial as well as epithelial expression sites. Note that exceptions to this rule, caused by pathological alterations, unknown individual factors, or in rat, by strain differences, might occur.

sclerosis and vascular restenosis [54–56]. In animal studies, it has been suggested that Ang II is involved in nephrogenesis and renal vascular development [57–59]. Angiotensins are also probably responsible for the development of glomerular sclerosis, interstitial sclerosis, and proteinuria in some diseases [58, 60, 61].

In this study, diabetes mellitus, for example, was significantly associated with a neoexpression of ACE in the glomerular tuft (Fig. 2C). This was confirmed in animal models [62] and was recently corroborated by others [63]. The onset and progression of diabetic nephropathy was found to be associated with the genetic polymorphism of ACE and elevated plasma levels of the enzyme [64, 65]. On the other hand, several studies in humans and animals indicate that ACE inhibitors have beneficial effects on diabetic nephropathy, and it has been proposed that these protective effects are independent of the reduction of blood pressure [66, 67]. ACE inhibitors reduce the progression of albuminuria and the degradation of renal function in type I diabetes and in incipient renal failure of several origins [62, 68–72]. The beneficial effects of ACE inhibition may be mediated by the inhibition of ACE, which is abnormally expressed by endothelial cells of the renal vasculature.

Up- and down-regulation or loss of ACE was observed in different non-neoplastic kidney diseases, as well as in different renal tissue compartments. Even in the complexity of an altered renal morphology, renal function, clinical presentation of patients, as well as therapeutical intervention, some of these changes of ACE expression were found clearly associated with certain pathological conditions: (*a*) fibrotic remodeling of renal tissues, (*b*) chronic arterial hypertension, (*c*) diabetes mellitus, (*d*) ischemic renal shock, and (*e*) acute pyelonephritis. Semiquantitative immunohistochemistry and the employed statistical method may both tend to incorrectly estimate the exact changes of functionally effective ACE molecules. However, as changes and interesting associations were detectable even using these relatively rough methods, we would like to point out that ACE is an important molecule in the pathophysiology of diseases affecting the human kidney. In the future, new technical approaches will help to study molecular changes more exactly even

on the level of single cells in normal as well as diseased tissues [73].

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