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Cathepsin B and L in nephron segments of rats with puromycin aminonucleoside nephrosis

CHRISTOPH J. OLBRICHT, JAMES K. CANNON, and C. CRAIG TISHER

Laboratory of Experimental Morphology, Division of Nephrology, Hypertension and Transplantation, University of Florida College of Medicine, Gainesville, Florida, USA

Cathepsin B and L in nephron segments of rats with puromycin aminonucleoside nephrosis. The intralysosomal proteinases, cathepsins B and L, were measured in microdissected segments of rat nephrons following a single injection of puromycin aminonucleoside (PAN). Z-Phenylalanyl-arginine-7-amido-4-methylcoumarin served as substrate. Enzyme activities, proteinuria, creatinine clearance and renal morphology were determined at specific time intervals following induction of PAN nephrosis. During the first three days following PAN injection, enzyme activities in S_2 and S_3 segments, protein excretion, creatinine clearance and appearance of the renal parenchyma resembled control animals. The enzyme activity in S_1 segments was slightly decreased, but returned to control levels at day six after injection. Days four through eight post-PAN injection were characterized by a dramatic increase in protein excretion and an increase in cathepsin B and L activity in S_2 and S_3 segments of the proximal tubule. During days 9 through 15 enzyme activity decreased significantly in S_2 segments despite continued proteinuria. Overt necrosis and cell injury were seen in the proximal tubule and probably account for the decrease in proteolytic activity. After day 15 following PAN injection, the level of proteinuria decreased, restoration of cathepsin activities occurred and a histopathologic picture of healing was present. The data suggest a positive relationship exists between stimulation of cathepsin B and L activity in S_2 and S_3 segments of the proximal tubule and increased protein filtration in PAN nephrosis. The increased enzyme activity reflects enhancement of the proteolytic capacity of the lysosomal system that is necessary for increased protein catabolism.

The nephrosis induced in rats by the administration of the aminonucleoside of puromycin (PAN) closely resembles the so–called "nil lesion" observed in humans. It is characterized by significant pathological alterations of the glomerular visceral epithelial cell and massive proteinuria. Later in the course of the disease, tubular damage occurs with vacuolation and degeneration of the epithelial cells [1–3].

Micropuncture experiments have demonstrated that the proteinuria is of glomerular origin [4–6]. The load of protein delivered to the proximal tubule is increased significantly, and the histopathology reveals evidence of increased endocytosis by the cells of the proximal tubule. The number of apical vacuoles and lysosomes is increased markedly [1, 2, 7, 8]. Large numbers of reabsorption droplets containing albumin and gammaglobulin are observed in the proximal tubule cells by immunofluorescence microscopy [8]. These findings are consistent with increased uptake of protein by the proximal tubule in PAN nephrosis. In normal rats approximately 10% of albumin catabolism occurs in the kidney [9], while in PAN nephrosis the kidney becomes a major site of albumin breakdown [10].

Only limited information is available currently concerning the proteolytic lysosomal enzymes involved in the increased protein catabolism that occurs in PAN nephrosis [11]. The cysteine proteinases, cathepsin B and L, are abundant in the kidney [12, 13]. Cathepsin B and L are also believed to be important in the turnover of cellular proteins, such as occurs during autophagy [14–20]. The highest activity has been found in S_1 and S_2 segments of the proximal tubule where under normal conditions about 70% of the filtered protein load is reabsorbed. In glomeruli and distal nephron segments where protein uptake is minimal under normal conditions, a comparatively low activity is found. This raises the possibility that cathepsins B and L are involved in intralysosomal degradation of reabsorbed proteins [13]. Therefore, the activation of cathepsins B and L may account for the increased protein catabolism observed in PAN nephrosis. To explore this hypothesis, we measured cathepsin B and L activities in microdissected nephron segments from rats with PAN nephrosis at specific time intervals following induction of PAN nephrosis. The results were interpreted in relationship to proteinuria, creatinine clearance and renal morphology at the specific time intervals.

Methods

Female Sprague–Dawley rats weighing 120 to 190 g were studied. The animals had free access to standard rat chow (Purina) and tap water.

Experimental protocol

Twenty–six rats were injected i.v. in the tail vein with a single dose of puromycin aminonucleoside (PAN) [6-dimethylamino-9 (3′amino-3′-deoxyribosyl) purine] (Sigma Chemical Company, St. Louis, Missouri, USA) amounting to 15 mg/100 g body weight as a 2% solution in saline. Another 15 control rats received an i.v. injection of the same volume (0.75 ml/100 g body wt) of saline only. The weight of the rats at sacrifice ranged from 143 to 260 g. The animals were kept intermittently in metabolic cages for serial 24 hour urine collections. In each animal the last collection period was 24 hour prior to sacrifice.

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The animals were sacrificed by stunning or by cervical dislocation and blood was withdrawn by cardiac puncture. The experimental rats were sacrificed at 1, 2, 2.5, 3, 4, 4.5, 5, 6, 6.5, 7, 7.5, 8.5, 9, 10, 11, 12, 13, 14, 15, 20, 21, 23, 28, 29, 31 and 34 days following injection of PAN. The 15 control rats were sacrificed 2, 4, 5, 6, 7, 9, 11, 12, 18, 22, 23, 24, 29, 30 and 34 days after injection.

Determination of creatinine and protein

Endogenous creatinine in urine and plasma was determined using a modified Jaffes picric acid method (Beckman Creatinine Analyzer 2, Beckman Instruments, Fullerton, California, USA). Protein in urine and plasma was determined by the Lowry et al method [21]. Urine proteins were precipitated with trichloroacetic acid and redissolved in sodium hydroxide.

Determination of cathepsin B and L activity in microdissected segments of rat nephron

The activities of cathepsins B and L in microdissected nephron segments were measured by an ultramicroassay developed recently in this laboratory [13]. We used Z-phenylalanyl-arginine-7-amido-4-methylcoumarin (Z-Phe-Arg-NMec) as the substrate for cathepsin B and L together [22]. The enzyme cleaves the carboxy end of arginine and releases the free 7-amino-4-methylcoumarin group which is highly fluorescent and can be measured at very low concentrations in small fluid volumes.

Solutions

All solutions were prepared from glass distilled water to minimize the background fluorescence. All chemicals were reagent grade. The dissection solution contained (in mmol/liter: NaCl, 136; KCl, 3; K_2HPO_4, 1; $MgSO_4$, 1.2; $CaCl_2$, 2; sodium lactate, 4; sodium citrate, 1; L-alanine, 6; and glucose 5.5). The collagenase solution which was prepared fresh daily was identical to the dissection solution except for the addition of 0.1% collagenase (Sigma, Type I). The rinsing solution contained (in mmol/liter): Na_2HPO_4, 11.5; KH_2PO_4, 55.2; and EDTA, 4. The pH was 6.0 and the osmolality was 141 mOsm/kg H_2O. The preincubation solution was the same as the rinsing solution but, in addition, contained 0.2% Triton × 100 (Sigma) and 0.05% bovine serum albumin (Sigma). The substrate, Z-Phe-Arg-NMec, was obtained from Bachem Feinchemikalien, Bubendorf, Switzerland. A 10 mmol stock solution of the substrate was prepared in DMSO (dimethyl sulphoxide). This stock solution was diluted daily to a 1 mmol solution with 0.1% Brij 35 solution (Sigma). To this final substrate solution, 8 mmol/liter cysteine were added immediately before use. The stop solution contained 100 mmol/liter iodoacetate in a buffer containing 30 mmol/liter sodium acetate and 70 mmol/liter acetic acid. The pH was 4.75. For preparation of standard curves, the product 7-amino-4-methylcoumarin (NMec) was obtained from Bachem Feinchemikalien, Bubendorf, Switzerland.

Collagenase treatment

Immediately after sacrifice the left kidney was removed rapidly and immersed in ice–cold dissection solution. Twenty ml of the same solution was perfused through the renal artery to remove all blood remaining in the kidney. This was followed by perfusion of 10 ml of the ice–cold collagenase solution. Pieces of tissue were then sliced along the corticomedullary axis and incubated for 60 minutes in the same collagenase solution gassed with 100% 0_2 in a shaking water bath at 37°C. The tissue was then rinsed with the dissection solution and maintained on ice until dissection. Collagenase was used to facilitate dissection of the proximal tubule segments. In preliminary experiments performed on rabbit proximal tubule segments, the collagenase treatment did not alter cathepsin activities significantly [13].

Microdissection

Individual tubules were dissected as described previously by Burg and Orloff [23]. Nephrons with at least one segment of proximal tubule on the surface were identified as superficial and only superficial nephrons were dissected. Based on morphology and function three segments of the proximal tubule, S_1, S_2, and S_3, can be identified and dissected separately [24, 25]. In the present study S_1 was identified as the first 1.5 mm of the proximal tubule attached to the glomerulus, while S_2 included the last 1.5 mm of the pars convoluta, and S_3 was identified as the last 1.5 mm of the pars recta immediately before the transition into the thin descending limb of Henle. The dissected segments were straightened and the length was measured using an eyepiece micrometer at 30 × magnification. The length varied from 200 to 1500 μm. Each segment was then transferred into a second dissection dish with the rinsing solution. After 10 seconds the segments were transferred with a 2 μl volumetric pipette into a reaction vial containing 25 μl of preincubation solution. The vials were then sealed and frozen in dry ice for 10 minutes before preincubation was initiated. In each rat between three and ten samples of each segment were dissected and analyzed.

Incubation

The samples were preincubated for 10 minutes in a shaking water bath at 37°C. Next, the vials were kept on ice while 25 μl of substrate solution were added. At this point the samples were incubated for 60 minutes in a shaking water bath at 37°C. The enzyme reaction was stopped by placing the vials on ice and adding 500 μl of stop solution. To evaluate the background fluorescence due to either substrate contamination with reaction product or spontaneous hydrolysis of the substrate during incubation, vials containing 25 μl of preincubation solution and 25 μl of substrate solution without nephron segments were treated identically. A standard curve was measured with each experiment.

Measurement of fluorescence

The fluorescence was measured with a Turner Model III fluorometer (Turner Assoc., Palo Alto, California, USA) using the reaction vial as a cuvette. The sample was excited at 365 nm (Corning filter CS-7-83) and the emission was recorded at 450 nm (Corning filter CS-3-72). The fluorometer reading was adjusted to zero with glass distilled water. Enzyme activity was calculated as pmol of NMec generated per mm tubule length per minute from the standard curve using NMec.

In vitro inhibition with PAN

To evaluate the possible effect of PAN on cathepsin B and L activities in vitro, we incubated isolated tubule segments with PAN. Seven S_1 segments dissected from one female rat were incubated without and eight S_1 segments with 1.0 mmol/liter PAN. Eight S_1 segments from a second female rat were incubated without PAN and eight S_1 segments were incubated with 3.0 mmol/liter PAN.

Histologic studies

The kidneys of nine female Sprague–Dawley rats of the same age and weight as those used in the biochemical studies were examined by light microscopy for evidence of tubular injury following PAN injection. Seven animals were injected in the tail vein with the single dose of PAN (15 mg/100 g body wt) as a 2% solution in saline. They were examined at 2 ($N = 2$), 14 ($N = 3$), and 28 ($N = 2$) days after injection. The two remaining animals received an i.v. injection of the same volume (0.75 ml/100 g body wt) of saline only and served as controls.

At the time of sacrifice, the animals were anesthetized by intraperitoneal injection of pentobarbital (35 mg/kg body wt) in preparation for intravascular perfusion fixation of the kidneys as described by Maunsbach [24]. After cannulation of the abdominal aorta, an isotonic sodium chloride solution was infused for two to three seconds for removal of the blood, followed by perfusion of the kidneys for three minutes with a 2% solution of glutaraldehyde buffered in 0.1 M sodium cacodylate with a pH of 7.4 and an osmolality of 430 mOsm/kg H_2O. The perfusion pressure was maintained at 120 mm Hg.

After completion of the perfusion fixation, the left kidney was excised and immersed in the same fixative for an additional three hours in preparation for light microscopic examination. Random blocks of tissue were taken from the outer and inner cortex for evaluation. The tissue was rinsed in 0.1 M sodium cacodylate buffer containing 7.5% sucrose and 2% NaCl, postfixed in 2% OsO_4 in a cacodylate buffer for one hour, dehydrated in graded ethanol solutions and propylene oxide, and embedded in Epon. One micrometer thick sections were cut from representative blocks of cortex and stained with toluidine blue for light microscopy.

Statistics

The significance of differences obtained for the same segments of the nephron from control and PAN-injected rats at different days was tested by using the two–way analysis of variance (ANOVA). Where the ANOVA was significant, Student's *t*-test was used to identify differences between controls and PAN-injected rats. The significance of differences between different segments of the control group was evaluated by Students *t*-test for paired data. The significance of differences in creatinine clearance was tested using two–way analysis of variance (ANOVA). *P* values <0.05 were considered significant.

Results

Protein excretion

The mean urinary protein excretion in the 15 control rats was 1.5 ± 0.8 mg/24 hr. There was a slight increase in protein excretion with time (Fig. 1). The level of proteinuria measured in the PAN-injected rats is shown in Figure 2. On day 5 there was a dramatic rise in the level of proteinuria. The maximum mean value of 340 mg/24 hr (range, 75 to 565 mg/24 hr) was reached at day 7 The urine protein excretion remained high through the 13th day after PAN injection and then declined progressively to near normal values by day 30.

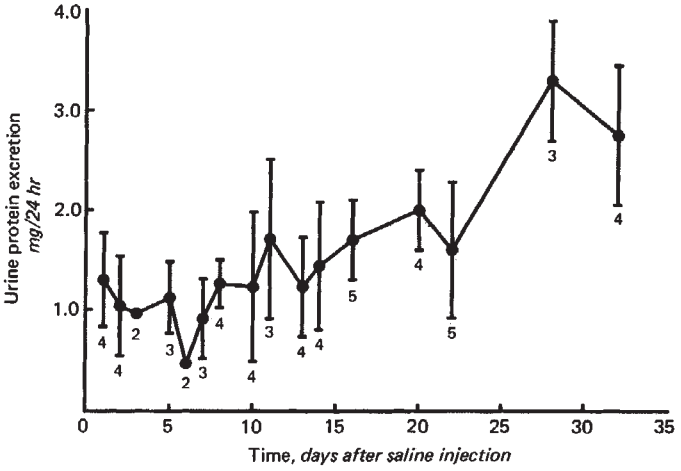

Fig. 1. *Twenty–four–hour protein excretion in control rats after a single intravenous injection of isotonic saline, 0.75 ml/100 g body wt.* The bars indicate the standard deviation around the arithmetic mean. The numbers below the bars indicate the number of urine collections at each time interval.

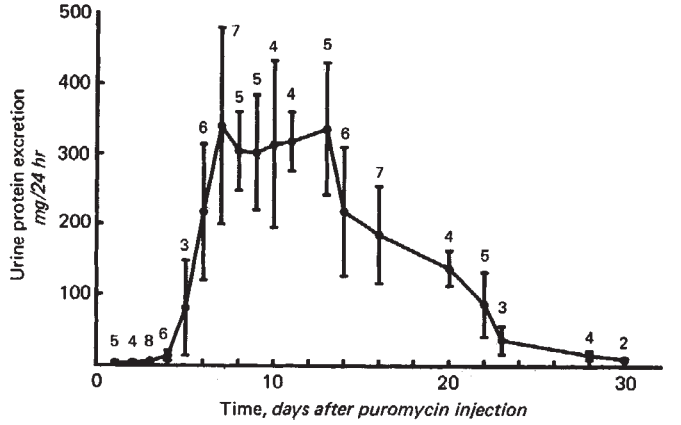

Fig. 2. *Twenty–four–hour protein excretion in PAN-Injected rats.* The bars indicate the standard deviation around the arithmetic mean. The numbers above the bars indicate the number of urine collections at each time interval.

Creatinine clearance

The endogenous creatinine clearance did not change with time in the saline–injected control group. It was 0.85 ± 0.14 ml/min/100 g body weight. Following PAN injection significant changes of the endogenous creatinine clearance occurred ($P < 0.001$). During the first five days after PAN injection the creatinine clearance was within or slightly below the control range (Fig. 3). A marked decrease in the clearance occurred two days after the onset of heavy proteinuria at day six. The

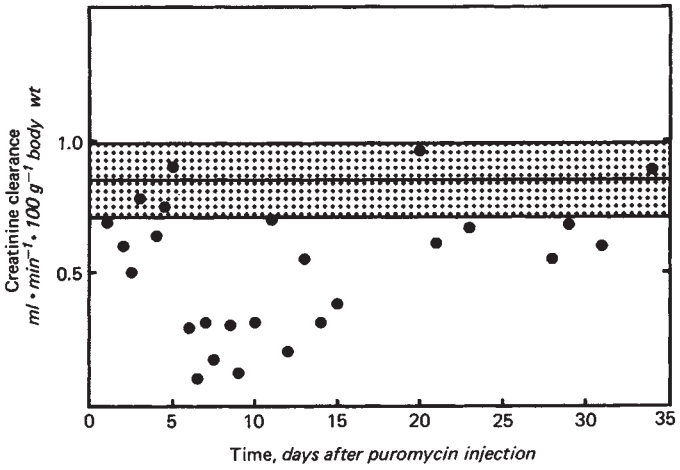

Fig. 3. *Endogenous creatinine clearance in control rats and in PAN-injected rats.* The shaded area indicates the mean ± SD of control values, $N = 15$. The creatinine clearance in controls did not change with time. The black dots indicate the creatinine clearances of PAN-injected rats at the day of experiment, $N = 26$.

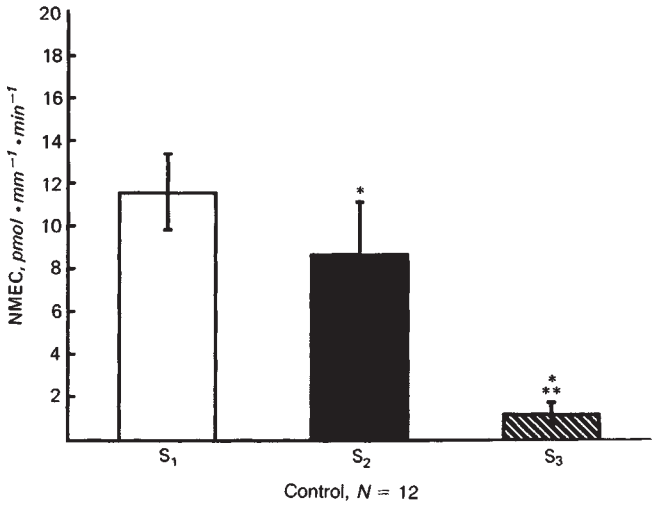

Fig. 4. *Cathepsin B and L activities in segments of proximal tubules of control rats.* The values are given as the mean ± SD. $N = 12$ rats. In each rat between 3 and 9 samples of each segment were analyzed. One asterisk denotes a significant difference from S_1 ($P < 0.05$). Two asterisks denote a significant difference from S_2 ($P < 0.001$).

creatinine clearance remained low until day 15. During this period the mean clearance was 0.31 ± 0.16 ml/min/100 g body weight. At day 20 the clearance increased toward values slightly below or within the control range.

Cathepsin activities in specific segments of proximal tubule

In control rats (Fig. 4), the cathepsin B and L activities in the three specific segments of the proximal tubule did not change with time following saline injection. The values were similar to those we have reported previously [13]. The activity was highest in the S_1 segments. The lower value in the S_2 segments was significantly different from the value for S_1 ($P < 0.01$). The S_3 segment had a comparatively low activity that did not exceed 17% of either the S_1 or S_2 activities.

The activities of cathepsins B and L in the S_1, S_2, and S_3 segments of PAN-injected rats are shown in Figures 5, 6, and 7, respectively. In all three segments the time following PAN injection had a significant impact on cathepsin B and L activities ($P < 0.001$ in all segments). In S_1 segments (Fig. 5), the time course of enzyme activities was significantly different from control rats ($P < 0.05$). The cathepsin B and L activity was below control values during the first six days after PAN injection. Between day six and day ten after PAN injection, most values were within the control range. Following the tenth day after injection no consistent change of cathepsin B and L activities was measured. Six values were significantly below control, five values were within the normal range and one activity was above control.

The cathepsin activities in S_2 segments are depicted in Figure 6. The time course of cathepsin B and L activities was different in PAN-injected rats and controls ($P < 0.001$). During the first three days following PAN injection a slight increase occurred from a subnormal value at the first day to normal values of cathepsin B and L on the second and third days after PAN injection. In animals sacrificed 4 to 7.5 days after PAN injection the cathepsin B and L activities were significantly higher in comparison to control values ($P < 0.05$, except on day 6). The

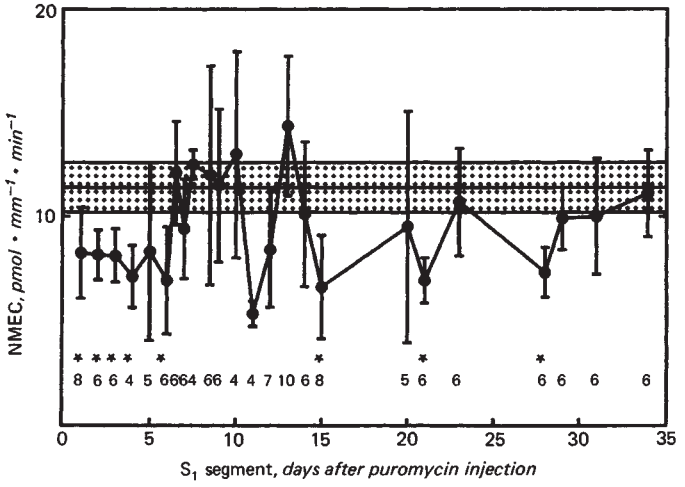

Fig. 5. *Cathepsin B and L activities in S_1 segments of control and PAN-injected rats.* The shaded area indicates the mean ± SD of the control values. $N = 12$ rats; in each rat between 4 and 8 S_1 segments were analyzed. The enzyme activities in S_1 segments of control rats did not change with time. Solid circles and bars indicate mean ± SD of values in individual PAN-injected rats. N = the number of segments analyzed per rat. Asterisks indicate a significant difference from control ($P < 0.05$).

increase in cathepsin activity coincided with the onset of heavy proteinuria. A sharp and consistent decrease of cathepsin B and L activities in S_2 segments occurred around the eighth day following PAN injection. The values were significantly below control activities through the 15th day after PAN injection. The cathepsin B and L activities observed in S_2 segments of rats sacrificed from 20 to 34 days after PAN injection were similar to control values.

In the S_3 segments the cathepsin B and L activity did not differ from control animals during the first six days following

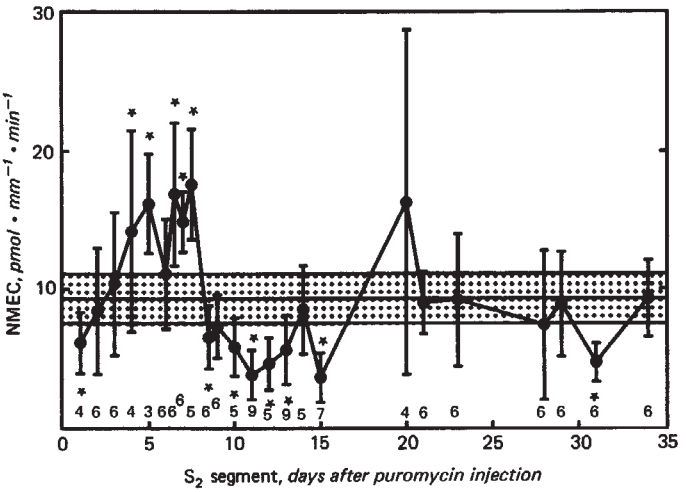

Fig. 6. *Cathepsin B and L activities in S_2 segments of control and PAN-injected rats.* The shaded area indicates the mean ± SD of the control values. N = 12 rats; in each rat between 3 and 9 S_2 segments were analyzed. The enzyme activities in S_2 segments of control rats did not change with time. Solid circles and bars indicate mean ± SD values in individual PAN-injected rats. N = the number of segments analyzed per rat. Asterisks indicate a significant difference from control ($P < 0.05$).

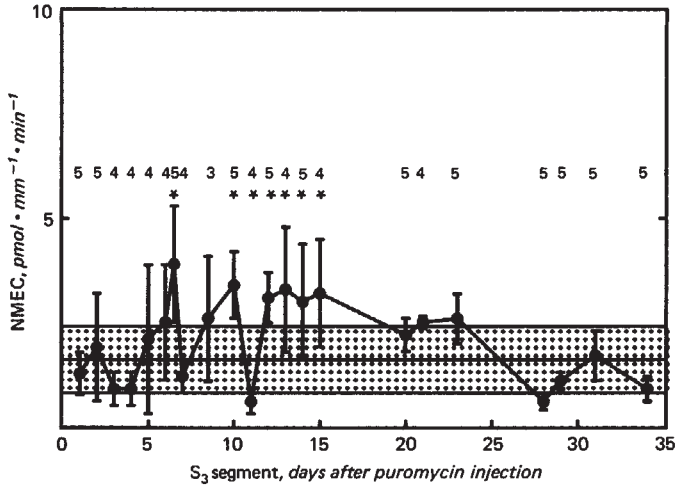

Fig. 7. *Cathepsin B and L activities in S_3 segments of control and PAN-injected rats.* The shaded area indicates the mean ± SD of the control values. N = 11 rat; in each rat between 3 and 5 S_3 segments were analyzed. The enzyme activities in S_3 segments of control rats did not change with time. Solid circles and bars indicate mean ± SD of values in individual PAN-injected rats. N = the number of segments analyzed per rat. Asterisks indicate a significant difference from control ($P < 0.05$).

PAN injection (Fig. 7). Later a slight increase in activity occurred and most values were above controls between day 6 and day 23 after PAN injection. A significant decrease of cathepsin B and L activity was observed in two animals at 11 and 28 days after PAN injection.

In vitro inhibition with PAN

The in vitro incubation of S_1 segments with PAN did not change cathepsin B and L activities significantly. After incubation with 1 mmol/liter PAN the enzyme activity was 8.3 ± 2.7 pmol/mm/min versus 10.3 ± 1.4 pmol/mm/min in control segments. After incubation with 3 mmol/liter, the enzyme activity was 13.1 ± 1.7 pmol/mm/min versus 15.2 ± 4.5 pmol/mm/min in control segments.

Histopathologic observations

Sections of the cortex from the control animals revealed excellent overall cellular preservation (Fig. 8A). Capillary loops within the glomeruli were patent and there was no evidence of hypercellularity or cell disruption. The three segments of the proximal tubule were easily discernible and entirely free of abnormalities. The distal convoluted tubule and collecting ducts were normal in appearance. The vessels and interstitium were unremarkable.

Two days after intravenous injection of PAN the appearance of the renal parenchyma was not different from the control animals (Fig. 8B). Again, the glomeruli, renal tubules, vessels and interstitium were well preserved and free of injury. At 14 days after PAN injection, however, the histologic picture was considerably different (Fig. 8C). Both lethal (overt tubular necrosis) and sublethal cell injury with evidence of early regeneration were present in many but not all proximal tubules. Often it was not possible to identify positively the three specific segments of the proximal tubule. Many distal convoluted tubules and collecting ducts contained either homogeneous appearing casts or cellular debris and manifested focii of cell injury. Interstitial inflammatory cells were scattered throughout the cortex in increased numbers. Many glomeruli exhibited dilated capillary loops that often contained large numbers of erythrocytes and platelets. Vacuolization of mesangial and visceral epithelial cells was also observed in a small number of glomeruli. These histopathologic findings were consistent in all three animals that were studied. At 28 days after PAN injection the histopathologic picture resembled that observed in the control animals (Fig. 8D). An occasional focus of interstitial scarring was seen, but the major portion of the renal parenchyma was well preserved and not different in appearance from the control animals. Again, the histopathologic findings were the same in each animal.

Discussion

The results of the present study demonstrate changes of intralysosomal cathepsin B and L activity in selected segments of the rat proximal tubule during the course of PAN nephrosis. In S_1 segments an inhibitory effect of PAN nephrosis on cathepsin B and L activity seems to prevail during the first six days following PAN injection (Fig. 5). After the sixth day most values for enzyme activity were within the control range. PAN is known to inhibit protein synthesis [26]. Hence, the reduction of enzyme activity may be explained best by a slight inhibition of the de novo synthesis of cathepsin B and L. A direct inhibiton of cathepsin B and L activity by PAN seems to be less likely, since in vitro incubation of S_1 segments with 1 and 3 mmol/liter PAN did not change the cathepsin B and L activity. The recovery of cathepsin B and L activity following the sixth day may be due to decreasing concentrations of PAN in the kidney cortex [27] and recovery of de novo synthesis of the enzymes.

In S_2 segments the time course of cathepsin B and L activities was different. During the first four days following PAN injec-

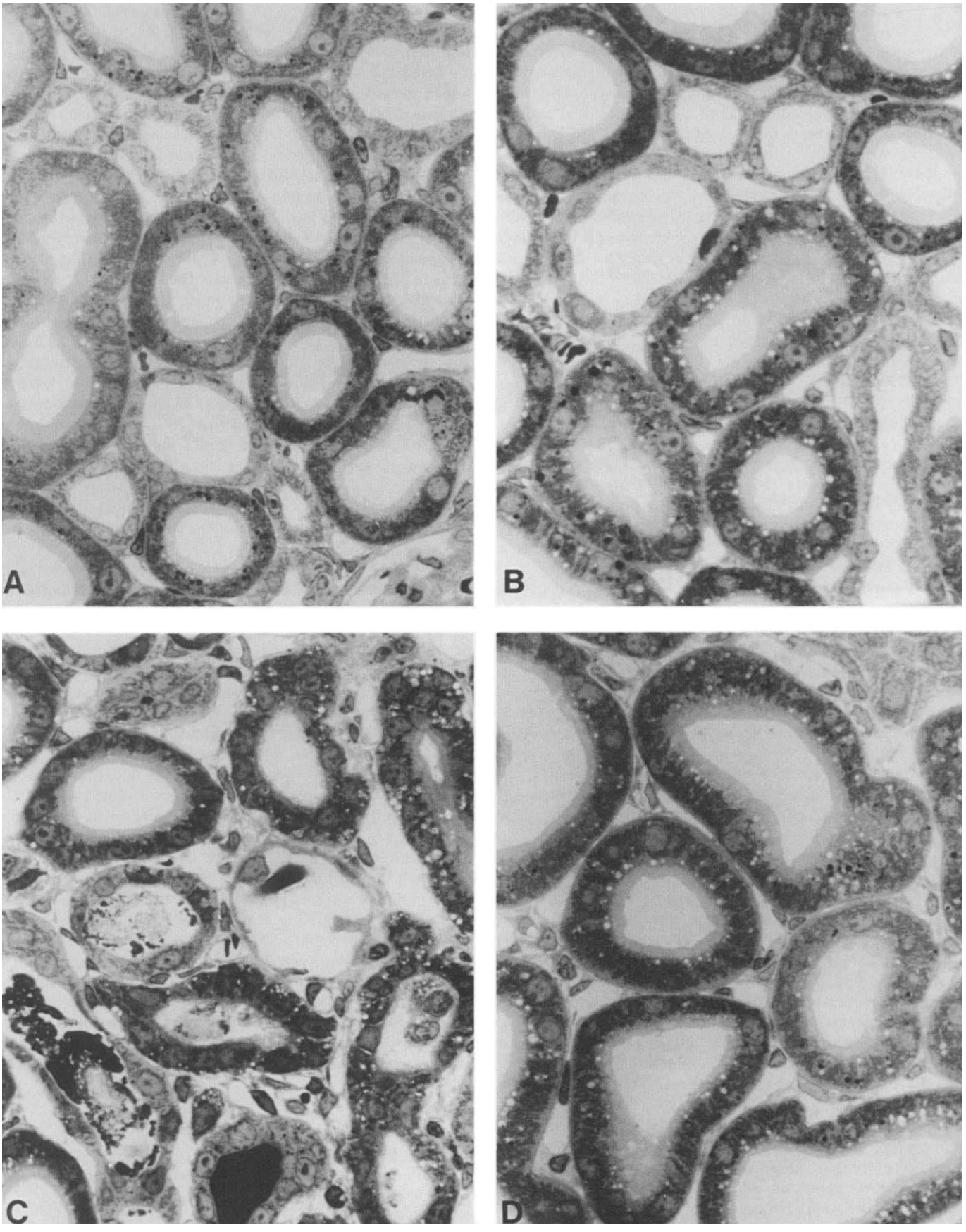

Fig. 8. *Photomicrographs illustrating appearance of renal cortex from control and PAN-injected rats:* ***a****. control animal;* ***b****. PAN animal at 2 days after injection;* ***c****. PAN animal at 14 days after injection; and* ***d****. PAN animal at 28 days after injection.* × 675.

tion, a continuous increase of enzyme activities occurred (Fig. 6) from a value below control levels on the first day to significantly elevated activities on the fourth day. Cathepsin B and L activity remained high until the eighth day after PAN injection. The significant increase of cathepsin B and L activity was accompanied by a dramatic increase in proteinuria. The sudden onset of proteinuria between day three and day five following PAN administration is quite characteristic of the single injection PAN nephrosis model described in Sprague–Dawley rats [2, 8, 11]. The concordance between the increased cathepsin activities and the proteinuria raises the likely possibility there was an increase in protein uptake by the S_2 segment in PAN nephrosis which was accompanied by an increase in activity of the intralysosomal proteolytic enzymes. Several arguments support this hypothesis. It is well established by micropuncture experiments that total protein load and the albumin load delivered to the early proximal tubule is increased significantly in PAN nephrosis and that the proteinuria is of glomerular origin [4–6]. The histopathology of proximal tubules at this stage of PAN nephrosis demonstrates increased numbers of apical vacuoles filled with hyaline material, and many proximal tubule profiles are characterized by cells containing increased numbers of lysosomes [1, 2, 7, 8]. Immunofluorescence studies have demonstrated that albumin and gammaglobulin constitute a substantial part of the hyaline material that is observed in the vacuoles and lysosomes, and the number of droplets containing albumin and gammaglobulin is increased with increased proteinuria induced with PAN [8]. Hence, there is considerable evidence that protein uptake by the proximal tubule is increased in the early phase of PAN nephrosis. The stimulation of cathepsin B and L activity is quite likely the result of this increased protein uptake. Additional support for this explanation comes from recent data by Baricos and Shah [11] who found increased activities of cathepsin D, another intralysosomal proteolytic enzyme, in homogenates of cortical tubule fragments from rats with PAN nephrosis, whereas the activity of alpha–fucosidase, a non-proteolytic enzyme of the lysosomes, was markedly decreased. In their study the stimulation of intralysosomal enzymes appeared to be confined to the proteolytic enzymes.

The failure to observe an increase in cathepsin B and L activity in S_1 segments during the same time period when activities in S_2 were increased appears to be contradictory to our working hypothesis. However, in recent experiments we observed a similar selective stimulation of cathepsin B and L activity in S_2 segments but not S_1 segments of rats with heavy proteinuria induced by repeated albumin injections [13]. In these experiments, the increase of cathepsin B and L activity in S_2 segments was higher than in rats with PAN nephrosis in spite of similar levels of proteinuria. This may be a further indication that the de novo synthesis of cathepsins B and L may be partially inhibited by PAN [26].

The decrease of cathepsin B and L activities in S_2 segments between day 8 and day 15 following PAN injection despite sustained proteinuria also seems contradictory to our working hypothesis that cathepsin activity is stimulated by increased tubular protein uptake. However, overt tubular necrosis as well as sublethal injury becomes evident in many proximal tubules during this phase of PAN nephrosis, and the drop in proteolytic activity probably reflects this cellular damage. It is also possible that due to increased protein uptake the lysosomes may be "overfed," exhaust and extrude their enzymes in the tubular lumen as suggested in a previous study [28]. Indeed, PAN nephrosis is associated with increased urinary excretion of lysosomal enzymes [29]. Urine excretion of nonplasma–derived proteinases and cathepsin D is increased significantly during the peak proteinuria of PAN nephrosis [11, 30]. However, a direct effect of PAN on protein uptake cannot be excluded at this stage of PAN nephrosis. Endocytosis and transport of albumin to the lysosomal system is a complex intracellular event that requires intact cell membranes and normal membrane interactions, a functional cytoskeleton and the biosynthetic source of metabolic energy [31]. PAN may cause defective protein uptake by disrupting one or more of these steps. The selective decrease of cathepsin B and L activity in S_2 segments suggests a higher susceptibility of this segment for either protein–related tubular damage or PAN-induced damage.

In S_3 segments the cathepsin B and L activity was increased between the sixth and fifteenth day following PAN injection (Fig. 7). A decrease of enzyme activity was not observed. As in S_2 segments the stimulation may be due to increased protein uptake by this part of the proximal tubule in heavy glomerular proteinuria. In recent experiments we observed a similar increase of cathepsin B and L activity in S_3 segments in rats with "albumin overflow" proteinuria [13].

The last phase of PAN nephrosis following the fifteenth day was characterized by a decrease in the level of proteinuria and restoration of cathepsin activities in all segments to levels that were not different from control animals. With the regeneration of the tubular epithelium, the cathepsin activities returned to normal values in the S_2 segments.

Whether or not the intralysosomal cathepsins play an important role in the pathophysiology associated with PAN nephrosis, as suggested by others [11], remains open. We believe our data suggest a relationship between stimulation of cathepsin B and L activities in the S_2 and S_3 segments of the proximal tubule and increased protein filtration. It is our hypothesis that the mechanism of stimulation involves an increase in protein uptake by the S_2 and S_3 segments. Although only 10% of albumin catabolism occurs in the kidney of normal rats, in PAN nephrosis the kidney becomes a major site of albumin breakdown [10]. The increase in cathepsin B and L activities appears to reflect activation of the proteolytic capacity of the lysosomal system that is necessary for the enhanced protein catabolism. Our inability to demonstrate an increase in cathepsin B and L activity in the S_1 segment in PAN nephrosis may indicate that endocytosis of endogenous proteins of a particular size and charge is already maximal under normal conditions in this region of the proximal tubule. We observed the same lack of response in animals made proteinuric with albumin loading and with aging [13].

Acknowledgments

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Reprint requests to Dr. C. Craig Tisher, Division of Nephrology, Hypertension and Transplantation, Box J-224, JHMHC, University of Florida, Gainesville, Florida 32610-0224, USA.

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