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Neutrophil-mediated post-ischemic tubular leakage in the rat kidney

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Neutrophil-mediated post-ischemic tubular leakage in the rat kidney. Neutropenia was induced in male Sprague-Dawley rats by administration of antineutrophil serum (ANS). A control group received an equal volume of inactive serum. After 45 minutes of unilateral complete renal ischemia the renal blood flow (RBF) was measured by an electromagnetic flow meter. The net filtration force (NFF) in glomerular capillaries, single nephron filtration rate (SNGFR) and frequency of tubular obstructions were estimated by a micropuncture technique. Tubular leakage was measured from the fractional recovery in the normal contralateral kidney of ^{3}H- or ^{14}C-inulin injected into surface proximal and distal tubules of the post-ischemic kidney. Neither ANS nor inactive serum had any influence on inulin clearance (C_{in}) in the normal kidney. In the post-ischemic kidney, C_{in} was four times higher in ANS-treated than in control animals. There was no difference in RBF, NFF, SNGFR or the frequency of tubular obstructions between neutrophil-depleted and control animals. The transtubular leakage of inulin injected into proximal tubules was substantially less in the ANS-treated than in the control group (11.3 ± 1.5% vs. 35.1 ± 6.5%; $P < 0.01$). But distal tubular leakage was equal in the two groups. The control group showed isosthenuria (350 ± 29 mOsm · kg^{-1}), while ANS-treated animals produced hyperosmolar urine (555 ± 60 mOsm · kg^{-1}; $P < 0.05$). It is concluded that neutrophil granulocytes mediate post-ischemic tubular leakage, which contributes to the depression in renal clearance parameters and the inability to produce hyperosmolar urine.

There is evidence to suggest that neutrophil granulocytes may act as mediators of ischemia-reperfusion injury [1]. Neutrophil depletion has thus been found to blunt capillary leakage resulting from ischemia-reperfusion in intestinal [2] and renal peritubular capillaries [3]. In the latter study, performed in our laboratory, we observed that neutrophil depletion also was associated with better preserved renal clearance parameters after ischemia. It has also recently been reported that addition of neutrophils to the perfusate accentuates ischemia-reperfusion injury in the isolated perfused kidney [4].

In the rat kidney, 45 minutes of ischemia result in a major decrease in the glomerular filtration rate (GFR), which is primarily caused by tubular obstructions [5], while alteration in renal hemodynamics and glomerular ultrafiltration coefficient (Kf) seem to play secondary roles [6, 7]. It is also evident that damage to the tubular epithelial barrier might lead to back-leakage of filtered inulin, a phenomenon which, for obvious reasons, reduces the clearance of inulin and other substances of the same size [8–11].

The aim of the present study was to investigate whether neutrophil granulocytes contribute to the functional deficit following renal ischemia. The results suggest that neutrophils, though not affecting determinants of GFR, mediate tubular leakage after ischemia-reperfusion injury.

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Methods

Surgical procedure

Experiments were performed on a total of 44 male Sprague-Dawley rats weighing 210 to 295 g (Möllegaard, Denmark). The animals had free access to water and standard rat chow (R3®, Evos, Södertälje, Sweden). They were anesthetized with Inactin® (Byk Gulden, FRG) injected intraperitoneally (i.p.) in a dose of 120 mg kg^{-1} body weight. The rats were tracheostomized and placed on a servo-controlled heating pad, which stabilized the body temperature at 37.5°C. Catheters were inserted into the left femoral vein and artery, the former for infusion of Ringer-bicarbonate solution at a rate of 5 ml kg^{-1} body wt hr^{-1} and the latter for monitoring of blood pressure and withdrawal of blood samples. The left kidney was exposed via a flank incision and immobilized in a lucite cup. A silicone catheter was inserted into the left ureter. The right ureter was exposed through a small suprapubic incision and cannulated with a polyethylene catheter.

Ischemia was evoked by occluding the left renal artery for 45 minutes, during which time the abdomen was closed with arterial forceps. After the end of the ischemic period the kidneys were allowed to recover for one hour before measurements began.

Neutrophil depletion

Neutropenia was induced by an i.p. injection of antineutrophil serum (ANS), given in a dose of 10 ml kg^{-1} body wt four hours before surgery. ANS was prepared essentially as described by Simpson and Ross [12]. Briefly, sheep were immunized with neutrophils harvested from the peritoneal cavity of Sprague-Dawley rats. The sheep serum was then absorbed against rat erythrocytes, rat lymphocytes and rat serum. Following precipitation with ammonium sulphate the serum was dialyzed and deep frozen until used. The control group received an equal volume of inactive serum (IS) collected from non-immunized sheep and subsequently prepared in exactly the same way as the ANS. The number of polymorphonuclear

leukocytes (PMNLs) in the blood was determined by a standard laboratory technique. Since the neutrophils represent over 97% of the total PMNL count in the blood, the values thus obtained essentially reflected the number of neutrophils.

Intrarenal neutrophil distribution

The intrarenal distribution of neutrophils in normal and post-ischemic kidneys was investigated in four rats treated with ANS and four treated with IS. After 45 minutes of unilateral (left kidney) ischemia and two hours of recirculation, both kidneys were removed, bled out, fixed in 10% formalin, embedded in paraffin and cut into 5 μm thick sections. The neutrophils were then visualized by a histochemical method, using a neutrophil-specific enzyme, chloroacetateesterase, as described by Moloney et al [13]. In a microscope with 400 times magnification the neutrophils were then identified as distinct red spots. By counting the red spots in a defined area of each section the distribution of neutrophils could also be quantified.

Renal blood flow and glomerular filtration dynamics

Renal blood flow was measured by an electromagnetic flow meter (MDL 503, Skalar, Holland), using a 1 mm wide probe placed around the left renal artery.

The hydrostatic pressure in the superficial structures was measured with the servo-nulling device described by Wiederhielm and coworkers [14]. The tubules were randomly selected as follows; the microscope was defocused and the glass capillary positioned just above the renal surface. After focusing the tubule just in front of the capillary was punctured. After ischemia three distinctly different types of tubule could be seen on the kidney surface. By observation of the transit of the dye lissamine green injected into the tubules, the difference between "obstructed" and "open" tubules could be distinguished. In the former we observed virtually no flow of lissamine green, whereas in the "open" tubules the transit time was rapid, that is, equivalent to a normal tubule. Pressure measurements confirmed the apparent distinction between the two tubule populations. The mean hydrostatic pressures in the "obstructed" ones were about twice as high as in the "open" tubules. A third category (collapsed tubules) could also be identified although these were rare.

The glomerular capillary pressure was measured by the indirect stop-flow technique as described by Gertz et al [15]. For this purpose only "open" tubules were used. Briefly, an early proximal tubule was punctured by a small glass capillary, and castor oil was then injected until the filtration ceased. The "stop-flow" pressure thus obtained was measured with a second glass capillary inserted proximal to the oil blockade. The glomerular capillary pressure was then calculated by adding the stop-flow pressure to the plasma colloid osmotic pressure of 19.3 mm Hg. The latter was calculated, using the formula of Landis and Pappenheimer [16], from the plasma protein concentration of 5.8% found here. The latter was measured by the method described by Lowry et al [17].

In a few animals the single nephron filtration rate (SNGFR) was estimated as the single nephron clearance of ^{3}H-inulin. For this purpose ^{3}H-inulin was added to the Ringer infusion, a 50 μCi bolus injection was given and followed by infusion of 50 μCi $\cdot$ hr^{-1} at a constant rate. After one hour of equilibration, castor oil was injected into an early proximal tubule and the tubular fluid was sampled for three minutes by gentle suction at a rate which kept the oil "block" in a fixed position. SNGFR were measured in "open tubules". The volume of the samples was measured from the length in constant-bore capillaries (0.5 μl, Drummond Scientific Company, USA). Plasma levels of ^{3}H-inulin were determined in blood samples drawn at regular intervals. The activity of ^{3}H in tubular fluid and plasma was measured by liquid scintillation technique.

To estimate the frequency of tubular obstructions, randomly selected tubules were categorized as "open" or "obstructed" by observation of the transit of lissamine green dye as previously described. The free-flow pressure was measured by the above servo-nulling device.

Microperfusion

Microperfusions was performed essentially as described by Donohoe et al [10]. For this purpose only tubules considered as "open" were used. By means of a microperfusion pump, ^{3}H- or ^{14}C-labeled inulin (127 and 77 cpm per nl, respectively) was injected into the tubules at a rate of 10 nl $\cdot$ min^{-1} through small glass capillaries with a tip diameter of 4 μm. At the same time the pressure in the perfused tubule was measured through another glass capillary inserted proximal to the perfusion capillary. To examine the possibility that the transepithelial leakage might depend upon the intratubular pressure, the tubules were randomized so that the average tubular pressure during perfusion was the same in the ANS- and IS-treated groups.

To identify distal tubules the dye lissamine green was injected into a proximal tubule through a glass capillary. After disappearing for a short period of time, the dye reappeared in a higher concentration and with a longer transit time in the distal tubule. A second glass capillary was used for perfusion of labeled inulin into the distal tubule, and the pressure in the corresponding proximal tubule was measured through the first capillary.

To minimize the relative influence of contamination during the puncture procedures a perfusion time of at least 10 minutes, and in most instances 20 minutes, was used. During the microperfusion and for at least two hours thereafter, urine was collected from both the left and right kidney. The percentage tubular leakage was then calculated from the recovery of perfused inulin from the contralateral kidney over that recovered from the two kidneys combined.

For determination of whole kidney inulin clearance (C_{In}) in these animals, non-radioactive inulin was infused at a constant rate of 1 mg $\cdot$ hr^{-1}. Blood samples were taken repeatedly for determination of the plasma inulin concentration. The concentrations of inulin in the urine and plasma were measured as described by Hilger, Klümper and Ullrich [18]. Urine osmolarity was determined by the freezing point depression method.

To check the reliability of the method, the leakage in two animals with intact kidneys was also determined. In both these cases less than 2% of the microinjected inulin was found in the contralateral kidney; thus the leakage in a normal kidney as measured by the present method was negligible.

Statistics

Results are given as mean $\pm$ 1 SE. Student's t-test was used to compare groups. A P value less than 0.05 was considered significant.

Table 1. Distribution of neutrophils in the renal cortex

	Post-ischemic kidney		Intact kidney	
	Located in glomerulus	Located outside glomerulus	Located in glomerulus	Located outside glomerulus
	$N \cdot \mu l^{-1}$ cortical tissue			
ANS	25 ± 4[a,b]	12 ± 4[a]	8 ± 1[a]	3 ± 1[a]
IS	1411 ± 216[b]	404 ± 115[b]	178 ± 22	99 ± 29

Values are means ± 1 SE. Number of neutrophils (N) in animals treated with antineutrophil serum (ANS) or inactive serum (IS).
[a] $P < 0.05$ compared with the IS group
[b] $P < 0.05$ compared with the intact (contralateral) kidney

Results

The PMNL count, which essentially reflects the neutrophils, in the blood was $0.04 \pm 0.01 \cdot 10^6 \cdot ml^{-1}$ in ANS-treated and $2.5 \pm 0.4 \cdot 10^6 \cdot ml^{-1}$ ($P < 0.001$) in IS-treated animals. In the kidney neutrophils were found mainly in the cortex and most frequently in the glomeruli. In the medulla the neutrophils were too few to allow a quantitative estimation. Table 1 summarizes the distribution of neutrophils within the renal cortex in the ANS- and IS-treated animals. The number of intrarenal neutrophils increased about sevenfold after ischemia in the IS group. The neutrophils were uniformly distributed between the glomeruli, and there was no observed difference, for instance, between the deep and surface glomeruli. In ANS-treated animals the intrarenal neutrophil content was substantially reduced.

It may be pointed out here that these figures probably represent an overestimation, since neutrophils could be sectioned and counted twice or more. However, with the greatest possibility, this error would be proportionally equal in all instances and would therefore not influence the relative distribution of neutrophils between or within the kidneys.

Renal blood flow and filtration pressure

The renal blood flow and the net filtration force in the glomerular capillaries are summarized in Table 2. No difference was found in these parameters between ANS- and IS-treated animals. SNGFR was also about the same in the two groups (30.2 ± 1.94 ($N = 12$) and 34.3 ± 2.4 ($N = 9$) $nl \cdot min^{-1}$, respectively). The tubular fluid to plasma ratio of inulin in the corresponding proximal tubules was 1.55 ± 0.15 in the ANS groups and 1.26 ± 0.08 in the group treated with IS; the difference was only of borderline significance ($P < 0.09$).

Tubular obstructions

After ischemia and reperfusion the kidney surface appeared heterogeneous some tubules were almost normal, while others were greatly dilated as a result of complete obstruction. As shown in Figure 1 the proportions of "open" and "obstructed" tubules were almost the same in the two groups. The average free-flow pressure (open and obstructed tubules combined) in the ANS group was 33.6 ± 1.5 mm Hg, which did not differ from the value of 32.6 ± 1.6 mm Hg found in the IS-treated animals.

Tubular leakage

Figure 2 shows the relation between intratubular pressure and inulin leakage in proximal tubules after ischemia. As expected, the leakage was more pronounced in tubules with high pressure. It is also evident from this figure that the leakage for a particular intratubular pressure was lower in ANS-treated than in IS-treated animals.

Table 3 summarizes the clearance of inulin in both kidneys and the recovery of microperfused inulin in the contralateral kidney.

The urine volume excretion and C_{In} in the contralateral kidney were equal in ANS- and IS-treated animals. After ischemia C_{In} was four times higher in the former than in the latter. This was due to a higher urine to plasma gradient of inulin in the ANS group than in the IS group (37 ± 7 and 11 ± 2, respectively, $P < 0.01$), while the urine volume excretion was equal in the two groups.

In the IS group isosthenuria was observed (urine osmolarity 350 ± 29) $mOsm \cdot kg^{-1}$). In contrast animals treated with ANS were able to produce hyperosmolar urine after ischemia (555 ± 60 $mOsm \cdot kg^{-1}$; $P < 0.05$), except for one rat, which produced diluted urine (160 $mOsm \cdot kg^{-1}$).

The total recovery (left + right kidney) of microinjected inulin in the ANS group was $86.5 \pm 3.3\%$, which was not different from the values of $84.1 \pm 5.0\%$ found in animals treated with IS.

Of the inulin injected into distal tubules, about 8% was recovered in the contralateral kidney and there was no difference between the two groups. In contrast, more than 35% of the inulin perfused into proximal tubules of the IS-treated animals was found in the urine from the contralateral kidney, as against only 10% in ANS-treated animals. This was not due to a difference in perfusion pressure.

It may be noted here that the fractional recovery in the contralateral kidney will underestimate the fractional leakage, as some of the inulin that leaks out to the circulation will return to the perfused kidney. From an inulin distribution volume of about 20% and the average total (left + right kidney) C_{In} of about 0.6 $ml \cdot min^{-1} \cdot 100\ g^{-1}$ body wt found here, the half-life of inulin will be 23 minutes and the two-hour sampling period will equal at least five half-lives of inulin. Hence, the amount of inulin that remains after two hours will be negligible, and the fractional leakage may thus be calculated by the formula:

$$\text{Fractional leakage} = \frac{M_{right} + M_{right} \cdot (C_{In}\ left/C_{In}\ right)}{M_{right} + M_{left}}$$

where M_{right} and M_{left} is the amount excreted in the two kidneys. The fractional leakage of inulin injected into proximal tubules will thus be $41.4 \pm 6.6\%$ in the IS group and $16.7 \pm 3.2\%$ ($P < 0.01$) in the ANS group.

Discussion

This study has shown that neutrophil depletion ameliorates the severe depression in inulin clearance that is observed after renal ischemia. Since the contralateral kidneys were not affected by the administration of either ANS or IS, that is, the values for C_{In} in these kidneys were in good accordance with those found in normal rats [5], the effects of neutrophil depletion were restricted to the ischemia-reperfusion injured kidney.

Table 2. Renal blood flow and hydrostatic pressure in superficial renal vasculature after 45 minutes of ischemia

	Renal blood flow *ml · min^{-1} 100 g^{-1} body wt*	Mean arterial	Glomerular capillaries	Welling point	Peritubular capillaries	Proximal tubules	ΔP
		mm Hg					
ANS (N = 10)	1.76 ± 0.05 (n = 15)	122 ± 7	64.0 ± 1.6 (n = 25)	24.2 ± 1.2 (n = 17)	17.6 ± 0.9 (n = 30)	27.8 ± 1.4 (n = 56)	≈ 16.9
IS (N = 11)	1.82 ± 0.11 (n = 12)	118 ± 9	61.8 ± 1.5 (n = 28)	22.6 ± 1.6 (n = 19)	18.2 ± 1.0 (n = 29)	25.9 ± 1.4 (n = 41)	≈ 16.6

Values are means ± 1 SE. Animals treated with antineutrophil serum (ANS) and inactive serum (IS). N = number of animals, n = number of measurements. The proximal tubular pressure refers to the open tubules. The net filtration force (ΔP) in glomerular capillaries was calculated by subtracting the mean proximal tubular pressure in "open" tubules and the colloid osmotic pressure (π) from the glomerular capillary pressure. The latter was measured only in "open" tubules.

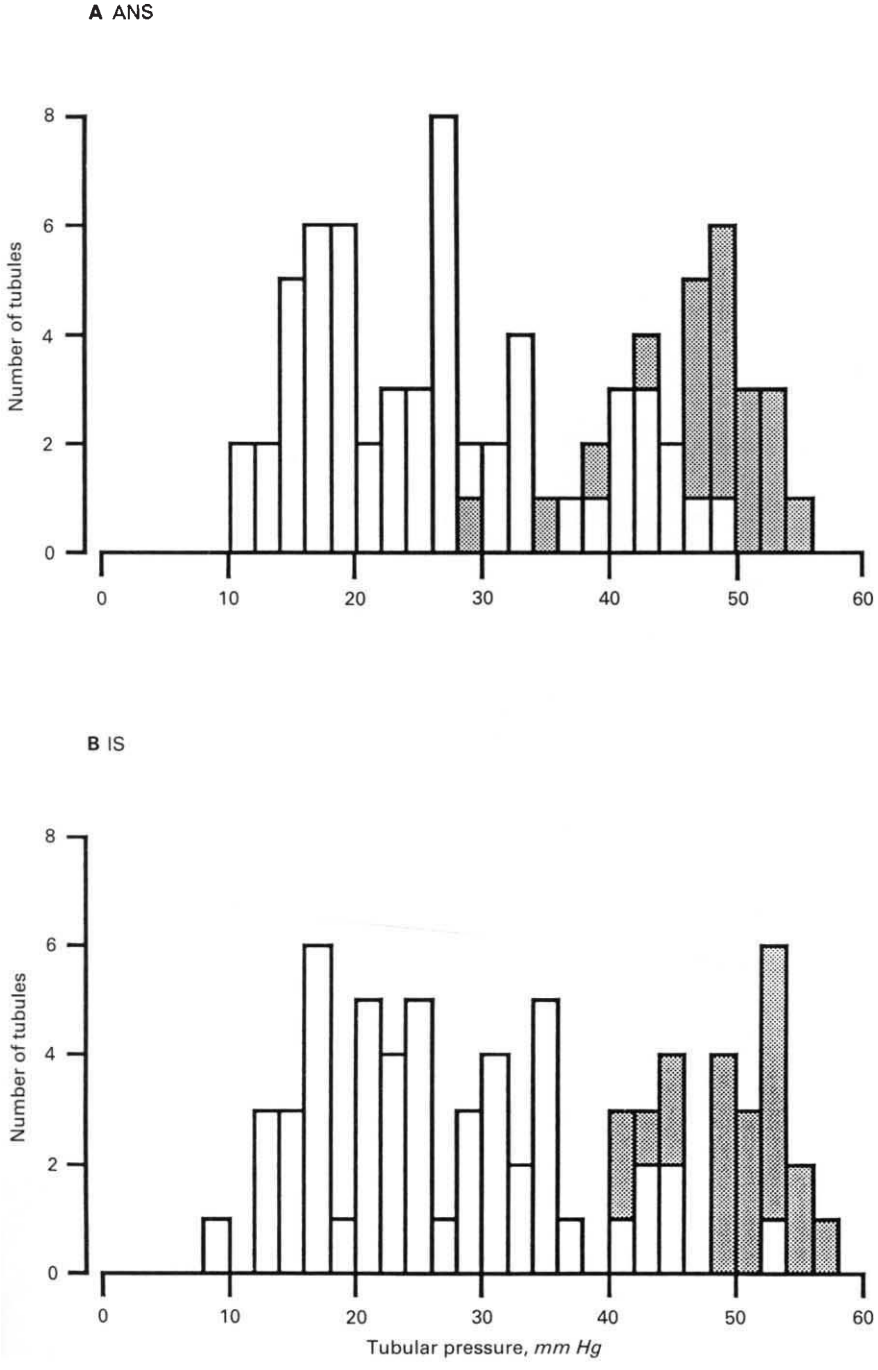

Fig. 1. *Frequency distribution of tubular pressures on the kidney surface after 45 min of ischemia and 1 hr of reperfusion in animals treated with antineutrophil serum (ANS) and inactive serum (IS).* Symbols are: (□) unobstructed tubules; (▨) obstructed tubules.

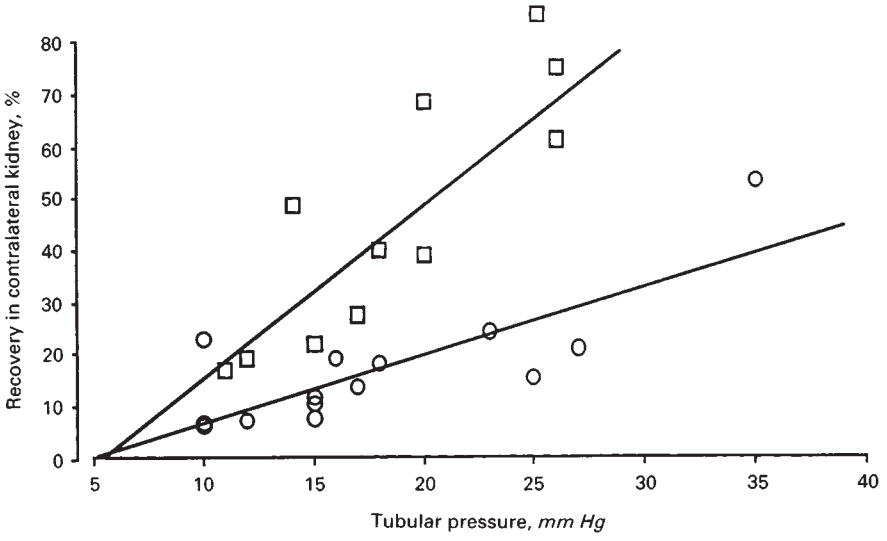

Fig. 2. *Relation between tubular pressure and inulin leakage after ischemia in ANS- (○) and IS-treated (□) animals.* Simple linear regression showed a significant correlation in both groups ($r^2 = 0.63$; $P < 0.01$ and $r^2 = 0.69$; $P < 0.001$ for the ANS and IS groups, respectively).

Table 3. Tubular leakage and urine excretion after ischemia

	Tubular leakage				Urine excretion					
	Proximal tubules		Distal tubules		Post-ischemic kidney			Contralateral kidney		
	Tubular pressure *mm Hg*	Recovery in contralateral kidney %	Tubular pressure *mm Hg*	Recovery in contralteral kidney %	V_U *μl · min⁻¹ 100 g⁻¹ body wt*	U/P_{In}	C_{In} *ml · min⁻¹ 100 g⁻¹ body wt*	V_U *μl · min⁻¹ 100 g⁻¹ body wt*	U/P_{In}	C_{In} *ml · min⁻¹ 100 g⁻¹ body wt*
ANS (N = 10)	18.4 ± 1.9 (n = 15)	11.3 ± 1.5[a]	14.0 ± 2.3 (n = 6)	8.0 ± 2.1	4.6 ± 0.8	37 ± 7[a]	0.17 ± 0.03[a]	1.9 ± 0.4	252 ± 40	0.51 ± 0.02
IS (N = 11)	17.9 ± 1.7 (n = 13)	35.1 ± 6.5	13.4 ± 1.9 (n = 6)	8.1 ± 2.8	4.9 ± 0.8	11 ± 2	0.04 ± 0.01	1.7 ± 0.2	301 ± 25	0.51 ± 0.05

Fractional recovery in the contralateral kidney of inulin microperfused into superficial tubules of the post-ischemic kidney in rats treated with antineutrophil serum (ANS) and inactive serum (IS). Urine output (V_U), urine to plasma ratio of inulin (U/P_{In}) and inulin clearance (C_{In}) in post-ischemic and contralateral kidneys. Values are means ± 1 SE. (N) indicates number of animals and (n) number of measurements.
[a] $P < 0.01$ compared with the IS group.

It also seems evident that the beneficial effect of neutrophil depletion was not exerted through influence on the determinants of glomerular ultrafiltration, as RBF and the driving force for glomerular ultrafiltration were the same in the ANS-treated and IS-treated animals. An effect on the the ultrafiltration coefficient (Kf) might possibly have been suspected, on the basis of the distribution of neutrophils in the glomerulus. However, the values for SNGFR obtained from proximal tubular fluid samples were equal in the two groups. Although SNGFR was measured in only four animals, the figures obtained were in good agreement with those previously found with the same ischemic model in animals that had not received any treatment [5]. It should be pointed out that during the sampling of tubular fluid for determination of SNGFR the tubular pressure is artificially reduced almost to control values. Hence, the finding that SNGFR was only slightly lower than in a normal kidney implies that the ultrafiltration process per se in both ANS- and IS-treated animals was fairly intact after ischemia.

It follows from the above reasoning that reduced tubular leakage is the most likely explanation for the better preserved C_{In} in neutrophil-depleted animals as compared with controls. In fact, the difference in the magnitude of tubular leakage found here was about the same as the difference in C_{In} between neutrophil-depleted and control animals.

There is substantial evidence that back-leakage of filtered substances across the tubular wall is a prominent feature after renal ischemia in both rats [9, 10] and humans [11]. The objection has been raised, however, that tubular leakage as demonstrated by intratubular microinjection could be an artifact due to an artificially induced increase in tubular pressure [19]. To circumvent this possibility we perfused only into open tubules. Moreover, the pressure was recorded throughout the perfusion procedure and no rise in tubular pressure during perfusion was found.

Donohoe and coworkers performed microinjections into obstructed tubules, also, and found that a substantial amount of the inulin was trapped within the kidney [10]. This is in contrast to the finding in the present study that in both ANS- and IS-treated animals more than 84% of the injected inulin was recovered within two hours. Hence, virtually all of the injected inulin had either been excreted by the perfused kidney or leaked back to the circulation and, little, if any was trapped in the kidney. Obviously, these results do not exclude trapping of inulin in areas of obstructed tubules, but they show that tubular

leakage occurs even in the absence of tubular obstruction. Although both tubular obstructions and leakage are symptoms of tubular injury the underlying mechanisms are not necessarily the same. The selective protection of the inulin barrier by neutrophil depletion found here may be explained by a neutrophil-derived permeability increasing factor, of which the neutrophils contain a large number of possible candidates [20]. Such a factor might interfere with the inulin barrier of renal tubules without causing tubular necrosis and sloughing of tubular components with subsequent obstructions.

The present finding that the tubular leakage was more pronounced in tubules with high pressure does not necessarily imply that there was more severe damage to the inulin barrier in these nephrons, but could merely be the consequence of a longer transit time in the high-pressure tubules resulting from partial obstruction.

It should be noted, however, that although the inulin clearance was four times higher in the ANS-treated than in the IS-treated, it was still only 20% of the normal. Thus, the present results are not incompatible with the concept that tubular obstructions are a major cause of depression of GFR in acute renal failure induced by renal artery clamping [5–7].

Mechanisms of neutrophil-mediated tubular injury

Most studies concerning neutrophil-mediated increase in permeability have dealt with endothelial cells. It has recently been demonstrated, however, that the inulin conductance of cultured intestinal epithelial cells increases during leukocyte transmigration [21]. It is also known that when activated, the neutrophils secrete reactive oxygen metabolites and a large number of enzymes and anionic proteins [20].

Since in the present experiments neutrophils were mainly accumulated in the glomeruli, the tubular leakage was probably not a direct consequence of migration of leukocytes across the tubular wall. It would rather seem that the impaired inulin barrier found here was caused by a substance secreted from the neutrophils. One possibility is that such a substance is secreted by neutrophils accumulated in the glomeruli and is subsequently transported to the tubular epithelium by the tubular fluid. Further, it seems reasonable to believe that the effect of such a substance might decline along the length of the tubule. This might explain why a neutrophil dependent leakage was observed in the proximal tubule and/or loop of Henle and not in the more distal part of the nephron.

However, the possibility of a vascular basis for the neutrophil-mediated tubular injury might also be considered. Thus, it could not be ruled out that a neutrophil derived factor might also influence the tubular epithelium from the apical side. It has also been shown that neutrophil depletion reduces the postischemic macromolecular permeability in the peritubular capillaries [3]. This post-glomerular vascular injury does, however, not seem to influence the post-glomerular resistance, since the values for renal blood flow and hydrostatic pressure in glomerular capillaries, welling points and peritubular capillaries were not different between ANS- and IS-treated animals.

It is also conceivable that the primary ischemic injury may enhance the susceptibility to, or act synergistically with, a neutrophil-derived factor, and that the location of the neutrophil-dependent leakage may reflect a more pronounced primary ischemic injury in this part of the nephron. Two "loci minores", both of which could explain the present results, have been demonstrated in the rat kidney, namely the straight segment of the proximal tubule and the thick ascending limb of the loop of Henle (TALH). The former shows most morphological damage [22], while in the latter the functional capacity is greatly impaired after ischemic injury [23, 24].

The unexceptional isosthenuria that follows renal ischemia has been proposed to be due to impaired medullary reperfusion [5] and a metabolic deficit in the medullary TALH [24]. However, consideration must be paid to the fact that the ability of the nephron to form either hyper- or hypo-osmolar urine also requires impermeability to water in the TALH. It is reasonable to assume that a neutrophil-induced leakage of inulin in TALH will also result in incomplete water restriction. This would explain why neutrophil-depleted rats managed to produce hyperosmolar or, as in one instance, hypo-osmolar urine, while isosthenuria was evident in the control group.

Interestingly, Hanley found that in isolated perfused rabbit tubules, that is, under conditions in which neutrophils are absent, the water impermeability in the TALH was intact after 60 minutes of ischemia [23]. Hence, ischemia per se may not influence the normal water restriction of the TALH, but the resulting release of some factor from an activated neutrophil may have this effect.

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