Peritubular capillaries of the renal cortex in experimental diabetes mellitus in the rat

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Summary

1. Microangiopathy affects the peritubular capillaries in experimental diabetes. Five to six months after streptozotocin administration to induce experimental diabetes in rats, a progressive increase of lymph flow and of the entry of albumin from the renal peritubular capillaries into the interstitium was seen.

2. Under these conditions, owing to the alteration of peritubular physical forces, the uptake of tubular reabsorbate into the capillaries can be impaired with potentially severe consequences in diabetic nephropathy.

Key words: albumin permeability, diabetic nephropathy, microangiopathy, peritubular capillaries, renal lymph flow.

Introduction

Nephropathy and progressive renal failure are major complications of juvenile-onset diabetes, and are related to a generalized microangiopathy common in this disease [1]. Findings of nodular or diffuse thickening of capillary basement membranes are typical. In advanced stages of the disease glomerular lesions described by Kimmelstiel and Wilson are characteristic [2].

The peritubular capillaries of the renal cortex comprise a surface area approximately 20-40 times that of the glomerular capillary bed [3].

Across the wall of the peritubular capillaries, reabsorbed tubular fluid flows inward from the peritubular interstitium. This process is governed by a balance of hydrostatic and colloid osmotic pressures, and it has been demonstrated that fluid uptake by the peritubular capillaries plays an important role in the overall control of tubular reabsorption [4, 5]. Quantitative estimates of the permeability of peritubular capillaries to macromolecules have been reported and it has also been determined that under normal conditions the reflexion coefficient of the reabsorbing peritubular capillary wall to albumin is near unity [6-8].

Parving & Rossing [9] showed an increased albumin permeability of the systemic circulation in human diabetic subjects. Since the effect of diabetes on the renal peritubular capillaries thus far has not been studied, and since an increased protein permeability of the peritubular capillaries could affect tubular reabsorption by altering the colloid osmotic pressure in the renal interstitium, we have examined the permeability of the peritubular capillaries to albumin in rats with streptozotocin-induced diabetes.

Preliminary reports related to this work have been published [10] and given at the Symposium: Lessons from Animal Diabetes, Jerusalem, Israel, 1982.

Methods

The rate of entry of plasma albumin from the peritubular capillaries into the renal cortical interstitium was measured by the method of Bell et al. [8] as a unidirectional clearance from the peritubular capillaries into the interstitium. This clearance, \( V' \), indicates the volume of plasma which contains a quantity of albumin entering the inter-
stition in 1 min. Because it is unidirectional, it differs from the widely used permeability x surface area (PS) product [11], which is a measure of the net clearance. Measurement of $\Psi$ by the method of Bell et al. [8] is based on the central volume principle [12], and involves two experimental determinations: the volume of distribution of albumin in the renal cortical interstitium, $V_i$, and the mean transit time of albumin through that distribution volume, $\bar{t}$.

Two groups of rats were injected intravenously with 45–65 mg of streptozotocin/kg at 6 weeks of age and measurements of $\Psi$ obtained after a period of 5–6 months for one group and 8–9 months for the other group. No insulin was administered throughout this study. Untreated littersmates served as controls.

In each group, animals were assigned to one of two experimental procedures, which were carried out under Inactin (K. Gulden, Konstanz, West Germany) anaesthesia. In one procedure, the mean transit time, $\bar{t}$, of tracer albumin from arterial plasma to renal lymph was determined. In these animals the femoral artery and vein were cannulated and a fine polyethylene catheter was inserted into a renal hilar lymph vessel. Albumin labelled with $^{131}$I as tracer was injected intravenously and the specific radioactivity of tracer-albumin versus time curves were obtained for both arterial plasma and renal lymph by frequent sampling. (Specific radioactivity was determined as a ratio of $^{131}$I-labelled albumin activity to steady-state $^{125}$I-labelled albumin activity. The latter tracer was injected about 3 h before the experiment [8].) Both curves were plotted and smoothed. Numerical deconvolution of the arterial (input) and lymph (output) curves was carried out to derive the impulse response function for the transport of tracer albumin from arterial blood plasma to renal lymph. This impulse response was obtained in the form of a probability density function (pdf) of transit times, and the mean value of this pdf was taken as the mean transit time, $\bar{t}$. Since flow through the cannula took 10–30 s, whereas $\bar{t}$ was in the order of 15–20 min, no correction was made for delay and dispersion of the tracer in the collecting cannula.

In the second procedure, the extravascular distribution volume of albumin in the renal cortex, $V_i$, was determined by calculating the difference between the total distribution volume, $V$, and the intravascular volume of distribution, $V_e$. Albumin labelled with $^{125}$I was injected intravenously and allowed to circulate over a period of approximately 3 h; then $^{131}$I-labelled albumin was injected and within 2–4 min the kidneys were removed and frozen in liquid nitrogen. While still frozen, slices of the renal cortex were cut, weighed and radioactivity was measured. The difference between the distribution volumes, $V - V_i$, was subjected to two corrections. First, extravasation of the $^{131}$I-labelled albumin was estimated as described by Bell et al. [8]. Second, filtered albumin present in the tubular lumina was excluded from the estimate of interstitial albumin pool. This was accomplished by using a highly significant positive linear regression of the difference $V - V_e$ (the dependent variable) on the urine albumin/plasma albumin concentration ratio, $U_{alb}/P_{alb}$ (the independent variable). Extrapolation to zero $U_{alb}/P_{alb}$ provided an estimate of the magnitude of the interstitial albumin pool under the condition when no albumin was present inside the tubules.

Both tracers used in these studies were commercially available labelled human serum albumin. When received from the supplier (Mallinckrodt Nuclear Corp., St Louis, MO, U.S.A.) these preparations contained 2–5% inorganic radioactivity not precipitated with 10% trichloroacetic acid in the presence of excess protein. By overnight dialysis at 4°C through cellophan, the inorganic radioactivity was reduced to less than 1% of the total. The equivalence of both $^{125}$I- and $^{131}$I-labelled preparations as tracers of plasma albumin was ascertained by injecting a mixture of both intravenously into a rat and observing the change in the ratio of radioactivities in the plasma over a period of approximately 3 h. Radioactivity was determined in a two-channel Packard well type automatic counter by accumulating at least 10,000 counts on each channel. Correction for $^{131}$I decay was applied when the time of all samples in one experiment exceeded 12 h. The unidirectional clearance of albumin from the peritubular capillaries into the interstitium, $\Psi$, was calculated as $V_e/\bar{t}$. Further details of the methods are given by Bell et al. [8].

Statistical comparisons between control and diabetic groups were done by $t$-test. When the variances of the groups compared were found to be different by the variance ratio ($F$) test, the degrees of freedom were adjusted to evaluate the $P$ value associated with $t$, as described by Bailey [13].

**Results**

Table 1 shows the results obtained in control and diabetic groups of animals at 5–6 months, and also at 8–9 months, after the administration of streptozotocin. Retarded growth, high urine flow and increased kidney weight were consistent features in the diabetic groups. Flow from a cannulated hilar lymph vessel was higher in the diabetic ani-
in the statistical evaluation are shown. Statistical significance between diabetic and control groups of the same age:

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<tr>
<th>Table 1. Experimental measurements in streptozotocin-injected diabetic rats at 5-6 months and 8-9 months after injection</th>
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<td><strong>5-6 months</strong></td>
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<tr>
<td>Body wt. (g)</td>
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<tr>
<td>Urine flow (ml/day)</td>
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<td>Kidney wt. (g)</td>
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<td>Lymph flow (μl/min)</td>
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<td>Lymph albumin/plasma albumin concentration ratio</td>
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<td><strong>i</strong> (min)</td>
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<td><strong>Vf</strong> (ml/100 g kidney wt.)</td>
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<td>10^6ψf (ml s⁻¹ 100 g⁻¹ of kidney)</td>
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Littermates served as controls. Means, SEM values and numbers of animals (n) included in the measurements are shown. Statistical significance between diabetic and control groups of the same age: *P < 0.05; **P < 0.01. For the measurement of Ψf in the bottom row [n] is indicated as the degrees of freedom used in the statistical evaluation (n₁ + n₂ - 2), where n₁ and n₂ are the number of measurements of Vf and i respectively, entering the calculation of Ψf. For definitions of symbols see the text.

Discussion
The results suggest a large and progressive abnormality in the renal postglomerular circulation in late experimental diabetes. The concurrent increases in Ψf and lymph flow demonstrate a large increase in the rate of entry of albumin from the peritubular capillaries into the interstitium. An increase in the protein permeability of the peritubular capillary wall was suggested by Miller & Michael [14], who observed an accumulation of albumin and IgG primarily at the external aspect of the tubular basement membrane.

The peritubular capillaries are not the only targets of diabetic microangiopathy: as noted above, Parving & Rossing [9] demonstrated an increased systemic transcapillary escape rate of tracer albumin in human juvenile-onset diabetic patients. Furthermore, Bollinger et al. [15] demonstrated a highly enhanced entry of fluorescent tracer from skin capillaries into subcutaneous interstitium in diabetic patients as compared with control subjects. In advanced diabetic retinopathy, increased extravasation of macromolecules is a common finding [16]. Proteinuria is also frequent in advanced diabetic patients, signifying an increased permeability of the glomerular capillaries [17].

Källskog & Wolgast [18] analysed the driving forces acting over the peritubular capillary wall after saline infusion, and found a slight increase in hydrostatic pressure and a substantial decrease in oncotic pressure in the subcapsular interstitial fluid. Both of these changes favoured a restoration of the net reabsorption driving force through the peritubular capillary membrane, but this driving force still remained, although slightly below the control level. In the scheme proposed by Källskog & Wolgast, fluid intake into the peritubular capil-
laries and, consequently, tubular reabsorption become fully restored only when extracellular fluid volume returns to the normal level. Recently, Pelayo et al. [19] presented a similar analysis of hypothetic consequences of a reduction in peritubular capillary reabsorptive permeability.

In contrast to temporary stimuli, diabetic alterations of the capillary wall are long-lasting and progressive, and increased entry of proteins into the renal cortical interstitium is also persistent. As shown by our experiments, in particular by the lymph albumin/plasma albumin concentration ratios, the primary response is in the direction of preserving the colloid osmotic pressure difference across the peritubular capillary wall, in exchange for increased interstitial volume and pressure. Increased interstitial volume in our experiments is implied by the increase of \( R_t \), the interstitial distribution volume of albumin. In biopsy material obtained from patients with diabetic nephropathy, the interstitium is often seen to be enlarged (M. Margittai, personal communication). In late diabetes these increases are permanent.

The long-range consequences of sustained elevations in interstitial volume and pressure are complex and in many ways unpredictable. Nevertheless, there are certain major implications which can be discerned: mild to moderate increases of the interstitial fluid pressure should tend to actuate an increase and potentially the interstitial distri-

The resulting disturbance in the balance of physical forces, which is responsible for the uptake of reabsorbed tubular materials into the peritubular capillaries, can eventually contribute to an aggravation of glomerular injury and to a rapid deterioration of renal function in diabetic nephropathy.

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References


