Proteinuria in the Acutely Diabetic Rat and its Response to Insulin Treatment

J. P. Pennell, M. M. Millard and M. H. Ashby

Department of Medicine, Division of Nephrology, University of Miami School of Medicine, Miami, Florida, USA

Summary. The development of proteinuria and its response to insulin therapy in acute experimental diabetes was examined in 11 female rats by sequentially measuring 24 h excretion of glucose and total protein before diabetes, during 5–37 days of acute streptozotocin diabetes, during and after 7–14 days of continuous subcutaneous insulin administration. Induction of diabetes promptly resulted in marked polyuria (78 ± 9 ml/24 h), and glycosuria (6.6 ± 0.7 g/24 h), while proteinuria quadrupled (from 4.7 ± 0.7 to 18.8 ± 1.8 mg/24 h, $p < 0.001$). Concurrent with amelioration of polyuria and glycosuria by insulin treatment, proteinuria decreased strikingly (8.5 ± 1.2 mg/24 h, $p < 0.001$). Polyuria and glycosuria resumed after discontinuing continuous subcutaneous insulin, and proteinuria promptly returned to pretreatment levels (19.0 ± 2.5 mg/24 h). Similarly decreased proteinuria (8.8 ± 0.9 mg/24 h) recurred in five rats retreated with insulin after 100 days of diabetes. Elevated proteinuria was not associated with lysozymuria or consistent changes in glomerular filtration rate. The rapid fluctuations in proteinuria attending acute diabetes and its effective treatment suggest that metabolic aberrations of diabetes may directly effect renal handling of proteins.

Key words: diabetes, proteinuria, glycosuria, insulin treatment, streptozotocin, glomerular filtration rate, lysozymuria

transplantation of pancreatic islet tissue [2–4] as well as in response to effective insulin therapy [5]. Similarly, in diabetic people with neither clinical renal disease nor clinical proteinuria, a decrease in urinary excretion of albumin has been demonstrated to occur in response to insulin therapy [6–8]. The rapidity of the decrease in proteinuria with effective insulin therapy in diabetic patients suggests that metabolic effects of diabetes may directly influence the renal handling of proteins [8].

The purpose of this study was twofold: 1) to determine how rapidly proteinuria develops after induction of experimental diabetes in rats and 2) to determine the response of proteinuria to short-term effective insulin therapy.

Methods

Eleven young adult female Munich-Wistar rats weighing 152 ± 4 g were maintained in individual metabolic cages so that urinary excretion of protein and glucose could be determined. They were allowed free access to standard laboratory diet and tap water. After permitting a few days for the rats to accommodate to the metabolic cages, 24 h samples of urine were collected for as many as 14 days. Then, after an overnight fast, diabetes was induced by means of IV injection of 50 mg/kg streptozotocin, and the rats were returned to the metabolic cages. Sequential 24 h samples of urine were collected for 4 days to 5 weeks. Then, either a 1-week ($n = 5$) or 2-week ($n = 6$) Alzet pump (Alza Corporation, Palo Alto) containing bovine soluble insulin in quantities sufficient to deliver continuously 2–4 units/day was implanted SC, and 24 h collections of urine were continued. After 7–14 days, the Alzet pumps were removed, and urine was collected for up to 4 weeks thereafter. Two months after the initial insulin treatment, six rats were administered insulin for a second time by means of an Alzet pump.

Standard renal clearance studies for measurement of glomerular filtration rate (GFR) were performed in six other conscious female rats before injection of streptozotocin and after 15–21 days of diabetes. The rats were surgically prepared for the clearance studies under light ether anaesthesia. Cannulae were placed in a

Proteinuria has emerged as the primary abnormality of renal function in rats with experimental diabetes [1, 2]. The level of proteinuria in chronically diabetic rats has been shown to decline in response to amelioration of the diabetic process by means of successful

Fig. 1. The mean 24 h excretions of urine, glucose and protein for the six rats administered insulin via 2-week Alzet pumps implanted on days 37 and 100 after streptozotocin-induction of diabetes. Data after day 80 are for the five of six rats exhibiting improvement in glycosuria in response to insulin. For proteinuria, the shaded area represents the standard error of the mean

jugular vein for infusions and in a femoral artery for sampling of blood and measurement of blood pressure. A soft silastic catheter was passed transurethorally into the bladder, and a snugly tied suture was placed circumferentially around the external urethra in order to secure the catheter and prevent leakage of urine. Rats then were placed in a plastic restraining cage and permitted to awaken and stabilize for 2–3 h. During that time, a priming dose of $8 \mu\text{Ci/ml}$ ^{14}C -inulin (New England Nuclear, Boston) was administered and followed by infusion of 0.154 mol/l saline containing $4 \mu\text{Ci/ml}$ ^{14}C -inulin at 0.03 ml/min . Additional volumes of 0.154 mol/l saline were infused so that the total rate of infusion was equal to the urinary flow rate. After urine flow had stabilized, at least three consecutive 10-min clearance periods were performed, with sampling of blood at the midpoint of each. The percentage deviation from the mean for GFR determinations in individual rats was $9.1 \pm 2.0\%$ (SEM), and the mean coefficient of variation was 11.6% . Upon completion of the clearance periods, the rats were again lightly anaesthetised with ether for removal of cannulae and surgical repair of the wounds, after which the animals were returned to their metabolic cages.

Analytical Methods

The 24 h samples of urine were analysed for volume, for glucose using a Beckman Glucose Analyzer (Beckman Instruments, Fullerton, California), and for total protein using the Coomassie blue dye-binding assay of Bradford [9] (Bio-Rad Laboratories, Richmond, California).

Measurements of urinary protein concentration were done in duplicate using internal standards. Protein assays were acceptable if duplicate values for a sample varied by less than 5% or if values for the internal standards were less than 5% from the known values. To evaluate whether glucose might interfere with the colorimetric determination for urinary protein, glucose was added to samples of normal rat urine in quantities sufficient to achieve a final glucose concentration of $0.139\text{--}1.39 \text{ mol/l}$. Analysis detected $100.8 \pm 8.7\%$ SD ($n = 10$) of the amount of protein present in the glucose-free urine. A concentrated glucose solution (2.78 mol/l) did not alter the baseline reading for the assay. Thus, glucose did not interfere with this assay for measuring urinary protein. To the extent that glycoproteins with a lesser affinity for binding Coomas-

sie blue would be increased in diabetic urine, the Bradford assay might underestimate total proteinuria in diabetic rats [10].

For determination of GFR, $10 \mu\text{l}$ samples of urine and plasma from the clearance periods were placed in a counting vial containing 10 ml of Aquasol (New England Nuclear, Boston) and counted for ^{14}C -inulin in a liquid scintillation counter (LKB Instruments, Rockville).

In order to assess whether streptozotocin-induced tubular toxicity might be contributing to proteinuria in acutely diabetic rats, samples of urine were obtained from six rats five days after induction of diabetes by IV streptozotocin and were analysed for presence of lysozyme using a radial diffusion technique (Kallestad Laboratories, Chaska, Minnesota).

The 24 h excretions of glucose and protein and the clearance of ^{14}C -inulin GFR were calculated using standard formulae. Statistical analyses were performed using Student's *t* test [11].

Results

The rapidity with which proteinuria develops after induction of diabetes was examined in 11 female rats. In five of these rats, one-week Alzet pumps were implanted on day 5 after induction of diabetes with streptozotocin. Two-week Alzet pumps were implanted in the other six rats on day 37 after induction of diabetes and again on day 100. Sequential mean urinary data for this subgroup are depicted in Figure 1.

Before being rendered diabetic, none of the rats exhibited glycosuria. Marked polyuria ($78 \pm 9 \text{ ml/24 h}$, $p < 0.001$) and glycosuria ($6.6 \pm 0.7 \text{ g/24 h}$, $p < 0.001$) developed rapidly after injection of streptozotocin. During the first five days of diabetes, the level of proteinuria more than doubled in each rat, and the mean values for all 11 rats increased from $4.7 \pm 0.7 \text{ mg/24 h}$ before diabetes to $13.9 \pm 2.6 \text{ mg/24 h}$ ($p < 0.001$). The six rats that were diabetic for five weeks before insulin treatment each showed further progressive increases in proteinuria (Fig. 1).

The continuous administration of insulin by means of a SC implanted Alzet pump successfully reduced the 24 h excretion of glucose to below 100 mg in all 11 rats. Glycosuria ranged from $0\text{--}100 \text{ mg/24 h}$ for 3–10 days in ten rats and from $45\text{--}450 \text{ mg/24 h}$ for 5 days in the eleventh rat. The 24 h urine volume returned to prediabetic levels in 9 of 11 rats and remained below 20 ml for 3–10 days in all rats. In conjunction with the marked decrease of both glycosuria and polyuria during insulin treatment, all 11 rats had a prompt and striking decrease in proteinuria (from 18.8 ± 1.8 pre-treatment to $8.5 \pm 1.2 \text{ mg/24 h}$, $p < 0.001$). Although the mean level of proteinuria during the period of insulin therapy (8.5 mg/24 h) was significantly greater than prior to induction of diabetes (4.7 mg/24 h , $p < 0.005$), each rat demonstrated a decline in pro-

teinuria to prediabetic levels at some point during insulin administration.

After the pumps were exhausted or removed, there was a prompt return of the marked glycosuria and polyuria, and proteinuria increased to, or above, pre-treatment levels.

Two-week pumps were implanted in six rats for a second time at 100 days after induction of diabetes. In five of six rats, there was a prompt decrease in glycosuria and polyuria accompanied again by a striking fall in the level of proteinuria from 22 ± 4 to 8.8 ± 0.9 mg/24 h ($p < 0.025$; Fig. 1).

Data for the six rats in which clearance studies were performed both before and after induction of diabetes are presented in Table 1. When diabetic, these rats demonstrated levels of polyuria, glycosuria and proteinuria comparable with the other 11 rats. In these six rats, there was no consistent directional change in GFR after induction of diabetes and development of proteinuria.

In six of the rats, lysozyme could not be detected in the urine collected 3–5 days after injection of streptozotocin, indicating the absence of lysozyme in concentrations exceeding 1.0 µg/dl.

Discussion

There were two striking findings in this study. First, the urinary excretion of protein increased promptly in conjunction with the development of glycosuria and polyuria in the acutely diabetic rat. Second, amelioration of the glycosuria and polyuria by means of insulin therapy resulted in a prompt and dramatic decline of proteinuria in rats with diabetes for as long as 3–4 months. These observations suggest that proteinuria in acute experimental diabetes may be a functional manifestation of acute metabolic aberrations of diabetes rather than the result of structural alterations of the kidney.

The data neither elucidate the mechanism whereby proteinuria occurred nor permit discrimination between glycosuria and polyuria as to which may be a primary determinant of the proteinuria. The possibility that glomerular haemodynamic factors contributed to proteinuria observed in this study seems remote since acutely diabetic rats demonstrated enhanced proteinuria in the absence of changes in GFR. Proteinuria has previously been observed to occur in chronically diabetic rats in the absence of altered GFR [2, 12]. The possible role of increased glomerular permeability as a mechanism for proteinuria in acutely diabetic rats has not been examined. Proteinuria in chronically diabetic rats has been shown to occur in the absence of altered clear-

Table 1. Metabolic and renal function data in six rats before and 15–21 days after induction of diabetes

	Normal	Diabetic	p^a
Body weight (g)	145 ± 3	152 ± 8	NS
Urine volume (ml/24 h)	7 ± 1	93 ± 10	<0.001
Glucose excretion (g/24 h)	0	7.6 ± 0.8	<0.001
Protein excretion (mg/24 h)	4 ± 1	24 ± 4	<0.005
GFR (ml/min) ^b	2.5 ± 0.2	2.6 ± 0.5	NS

Values are the mean $\pm$ SEM

^a Significance of the difference in individual rats, diabetic versus normal, using paired comparison. NS = not statistically significant ($p > 0.05$)

^b If a single rat with an unusually high GFR is excluded from statistical analysis, then the mean GFR ($\pm$ SEM) in five rats was 2.4 ± 0.1 ml/min before and 2.1 ± 0.1 ml/min after induction of diabetes. The difference in these paired measurements of GFR ($\sim 13 \pm 8\%$) was not statistically significant

ances of macromolecular neutral dextrans suggesting that the proteinuria did not result from increased size or number of glomerular pores [12].

Toxic damage of renal tubular cells could impair protein reabsorption or result in leakage of protein into the urine. Since streptozotocin used clinically may be a potent toxin to renal tubules [13] and since lysozymuria may accompany renal tubular damage [14], the urine obtained from proteinuric diabetic rats five days after streptozotocin injection was assayed for lysozyme, with negative results. The absence of lysozymuria suggests that renal tubules were intact and not contributing protein to the final urine.

One can only speculate regarding the biological significance of the rapid fluctuations in proteinuria attending acute diabetes and its treatment. The concept has long been advocated that functional microangiopathy precedes the characteristic anatomical changes of diabetes [15]. It is not known whether such a temporal sequence might indicate a cause and effect association or simply represent temporally distinct expressions of a common metabolic aberration. For instance, the morphogenesis of diabetic glomerulopathy has been attributed to increased extravasation of plasma proteins through glomerular capillaries and their accumulation in the mesangium [7]. On the other hand, it has been proposed that nonenzymatic glycosylation of proteins may provide the common link between hyperglycaemia and both the functional and structural abnormalities of diabetes [16].

In summary, proteinuria has been found to increase rapidly after induction of diabetes in rats and to decrease promptly in concurrence with the

amelioration of glycosuria by means of insulin therapy. Proteinuria was not associated with either lysozymuria or changes in GFR. Thus, the proteinuria of acute experimental diabetes appears to be an expression of a functional microangiopathy and to occur by mechanisms yet to be elucidated.

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J. Phillip Pennell
Division of Nephrology
Department of Medicine (R-126)
University of Miami School of Medicine
P. O. Box 016960
Miami, Florida 33101, USA