Prevention of Diabetic Glomerulopathy in Streptozotocin Diabetic Rats by Insulin Treatment

Albumin Excretion

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Summary. A single antibody radioimmunoassay has been used to measure albumin excretion in 3 groups of female Wistar rats. Two groups had streptozotocin diabetes and were treated daily with insulin for 6 months. In one of the diabetic groups good glycaemic control was attempted and throughout the 6 months plasma glucose levels were fairly close to normal (92 ± 33 mg/100 ml at 2300 h and 186 ± 9 mg/100 ml at 0800 h). In the other diabetic group poor control was intended and the group had consistent high plasma glucose levels (576 ± 89 mg/100 ml and 460 ± 43 mg/100 ml). The third group was a non-diabetic control group. – Albumin excretion was measured on two occasions: before the induction of diabetes and after 6 months of diabetes. The geometric mean albumin excretion increased from 0.38 to 2.56 mg/24 h in the 18 non-diabetic controls. In the 20 diabetic rats in “good” control the geometric mean albumin excretion increased from 0.37 to 1.58 mg/24 h (NS compared with controls) and in the group of 22 rats in poor control albumin excretion increased from 0.35 to 6.54 mg/24 h. – The increase in albumin excretion in rats in poor control differed significantly both from that of the non-diabetic controls ($2p = 0.023$) and from that of the “well-controlled” diabetic rats ($2p = 0.00011$).

Key words: Urine albumin in rats, radioimmunoassay, streptozotocin diabetes in rats, blood glucose control in rats.

membranes become thicker [13] and the mesangial regions enlarged, with increased amounts of basement membrane-like material [14]. If plasma glucose is kept at levels close to normal these changes in the kidney do not develop.

Albumin excretion is increased in diabetes in the rat, the increase being more pronounced the longer the diabetes duration [14]. The present study was undertaken to see if the increased albumin excretion in diabetic rats could be prevented by near-normalization of plasma glucose during a 6 month period.

Two diabetic groups were compared: one group with plasma glucose levels fairly close to normal and a second group with consistently high plasma glucose levels. A non-diabetic control group was also examined.

Material and Methods

Detailed description of streptozotocin diabetic rats, their body weight, streptozotocin administration, insulin treatment, urine collection procedure and urine glucose excretion has been given previously [11, 12, 13].

Three groups of female Wistar rats, aged 86 ± 12 days were used. Diabetes was induced by administering 90 mg streptozotocin IV. In one group of 20 streptozotocin diabetic rats good diabetic control was attempted – these are referred to as the ‘well-controlled’ diabetic group. In another group of 22 streptozotocin diabetic rats poor control was intended and this group is referred to as the ‘poorly-controlled’ diabetic group. The type of control was achieved by administration of different amounts of insulin to the two diabetic groups [11]. A special non-commercial very long-acting insulin (IV52-0) NOVO [16] was administered once a day. The insulin dose administered was governed by plasma glucose measured with Dextrostix® and an Ames Reflectance Meter (Ames Comp.) just before the insulin administration. Plasma glucose was measured in each animal every morning during the 6 months study except during week-ends. The two diabetic groups were compared to 18 non-diabetic controls. These animals had the same weight and age when the experiment started (Table 1).

In previous studies it has been shown that the kidney and the glomeruli become enlarged in rats with high plasma glucose concentrations maintained for 6 months [12]. Furthermore, glomerular basement

Table 1. Results from the 6 months experiment with groups of rats in different types of blood glucose control

	Non-diabetic	'Well-controlled' diabetic	'Poorly-controlled' diabetic
Number of animals	18	20	22
Body weight at start of experiment (g)	191±7	193±8	189±9
Body weight after 6 months (g)	314±30	320±28	224±29
Amount of insulin/day (U/kg)	0	7.8±2.3	1.6±0.2
Plasma glucose (mg/100 ml)			
0800 h	92±10 ^b	186±9 ^a	460±43 ^b
1100 h	—	118±50 ^c	342±70 ^c
2300 h	—	92±33 ^c	576±89 ^c
24 h urine volume (ml)	15±7 ^d	19±6 ^d	105±5 ^d
Urine glucose excretion per 24 h (g)	—	0.21±0.11 ^e	10±2 ^e
Albumin excretion before the induction of diabetes (mg/24 h)	0.38×/÷1.63	0.37×/÷1.76	0.35×/÷1.84
Albumin excretion after 6 months of diabetes (mg/24 h)	2.56×/÷2.37	1.58×/÷2.26	6.54×/÷2.86

Values are given as arithmetic mean ± SD except for albumin excretion where the geometric mean ×/÷ tolerance factor is given. The individual mean value has been used to calculate the mean of the group

^a 123 measurements in each animal (every Monday–Friday)

^b 12 measurements in each animal (one day/fortnight)

^c 2 measurements in each animal. After 3 and 5 months of diabetes

^d 184 measurements in each animal (at every day during the whole study)

^e 36 measurements in each animal (evenly distributed throughout the study)

The animals were kept in individual metabolic cages where urine was collected separately. Urine samples were examined from each animal on 4 consecutive 24 h periods, namely the last 4 days before the induction of diabetes and for another 4 day period after 6 months of diabetes. The individual mean of the 4 days was used to calculate the geometric mean value for the group.

A single-antibody radioimmunoassay was used to measure urinary albumin. The assay is a modification of the assay described by Miles et al. [8] for human urine albumin. The urine samples were stored deep frozen until analysis. After thawing they were diluted 1:25 to 1:200 with a 0.04 mol/l phosphate buffer (pH 8.0) containing 0.6 mmol/l sodium merthiolate with 0.05% bovine albumin (Ortho Diagnostics) to prevent adsorption of reagents to glassware [8]. The urines were then incubated overnight with iodinated rat albumin (Koch Light Laboratories) and rabbit anti-rat albumin (Nordic Pharmaceuticals and Diagnostics). Iodination of rat albumin was performed using the method described by Miles et al. [8]. Rat albumin, 500 µg in 100 µl buffer without bovine albumin, was mixed with 5 mCi of Na¹²⁵I (Amersham IMS 3) in 50 µl, to which 25 µl chloramine T (1 mg/ml) was added. The reaction time was 1 min whereafter 400 µl of sodium pyrosulphate (0.2 mg/ml) was added. The crude product of iodination was then separated on a Sephadex[®] column (Superfine G 200). The elution curves always exhibited 3 separate peaks. The second major peak contained the iodinated albumin. After incubation, microfiltration was performed through a 0.8 µm Millipore filter. The antibody bound activity retained on the filters was measured with a scintillation counter. Experiments on the reproducibility of the assay showed an average intra-assay coefficient of variation of 5.1% and an interassay coefficient of variation of 9.1%; the results of the recovery experiments after the addition of various amounts of rat albumin averaged 103 ± 6%.

The sensitivity of the radioimmunoassay was such that dilu-

tions of the urine samples of 1:25 to 1:200 were necessary. This implies that possible interference in the radioimmunoassay by variations in pH and solute concentration was excluded or at least greatly diminished.

The cross reactivity of the antialbumin serum was studied in experiments where large amounts of α, β and γ globulins were added. There was no decrease in binding percentage until concentrations were reached far exceeding those possibly obtaining in urine.

All urines were tested several times during the second and third month of diabetes to ensure that the poorly controlled rats had not developed haematuria (Hemastix[®], Ames).

Statistical Methods

Student's t-test for independent samples was used to compare the rise in albumin excretion between groups. Student's t-test for paired samples was used to compare the albumin excretion before the induction of diabetes to that after 6 months of diabetes within the groups. The mean value in the groups is the geometric mean, due to log-normal distribution of the data. Tolerance factor is the analogue of standard deviation and was used here because the mean values are geometric means. The mean values have to be multiplied or divided by the tolerance factor in order to obtain the tolerance interval in analogy to the more usual one: mean ± SD.

Results

The degree of glycaemic control during the 6 month period is shown in Table 1. In the 'well-controlled' diabetic rats the plasma glucose values measured at

0800 h just before insulin administration were nearly always the highest values of the 24 h period [12]. The plasma glucose levels at 1100 h and 2300 h were in the normal range. In the 'poorly-controlled' group plasma glucose levels were consistently high (Table 1). Glucose excretion was low in the 'well-controlled' rats 0.21 ± 0.11 (SD) g/24 h and on average the rats were without glucosuria on 57% of all days; in the 'poorly-controlled' group of diabetic rats daily mean glucose excretion was 10 ± 2 g.

Albumin excretion was the same in the three groups before the induction of diabetes at about 0.37 mg/24 h. After six months a significant increase in albumin excretion was seen in all three groups. In the non-diabetic controls, albumin excretion increased sevenfold ($2p = 10^{-6}$). In the 'well-controlled' diabetic rats albumin excretion increased fourfold ($2p = 4 \times 10^{-8}$), however, there was no statistically significant difference between the increase in albumin excretion in the two groups ($2p = 0.40$). In the 'poorly-controlled' diabetic rats the mean excretion of albumin was increased by a factor of 19 ($2p < 10^{-8}$). This was a significantly larger increase in albumin excretion when compared to the controls ($2p = 0.023$) and to the 'well-controlled' diabetic rats ($2p = 0.0011$) (Table 1).

When albumin excretion was expressed per 100 g body weight a significant increase in albumin excretion was still found between the groups as described above.

The investigations for blood in the urine showed that the 'poorly-controlled' rats did not develop haematuria. Only on very rare occasions were positive reactions seen in animals from all 3 groups.

Discussion

This study has demonstrated that normal albumin excretion can be preserved in experimental diabetes when strict control of plasma glucose has been maintained over a period of 6 months. On the other hand, poor diabetic control results in an increased albumin excretion compared to normal as well as to 'well-controlled' rats.

The results obtained by Hägg [2] are similar to ours. He found a significant increase in albumin excretion with age in male rats of the R-strain, Karolinska Institutet, Stockholm, using a modified Lowry technique. The method was not sensitive enough to measure albumin excretion in female rats. The increase in the male rats was from 4.1 to 29.3 mg/24 h from the age of 3 to 12 months.

The urinary albumin content was larger than in the present studies but that could be due to differ-

ences in sex and methods. Hägg furthermore showed that diabetic male rats have a significantly greater albumin excretion after 12 months of diabetes compared to a control group. This is in accordance with the present studies where diabetic rats in poor control were found to have larger albumin excretion.

Mauer et al. [7], using a radial immunodiffusion assay, have shown that diabetic, inbred Lewis male rats excrete about 3–4 times as much albumin per 100 g rat as non-diabetic controls after 7 months of diabetes. This is in agreement with the present studies where the increase in albumin excretion in the diabetic rats is of the same magnitude when calculated in the same way. The albumin excretion in the non-diabetic controls at the age of 3 months is also about the same in the two studies. Mauer et al. transplanted pancreatic islets to the streptozotocin diabetic rats, resulting in a cure of the diabetic state and followed by a significant fall in albumin excretion within one month. After 2 months albumin excretion was not different from that of a control group.

PAS stained sections studied by light microscopy have shown abnormalities in the kidneys of Lewis rats after 6 months of diabetes [6]. In our laboratory Ørskov et al. [20] showed changes at the light microscopic level only after 12 months of diabetes in Wistar rats of the strain used in the present study. In 16 of the 60 rats of the present study no light microscopic changes were observed after 6 months of diabetes when compared to 8 non-diabetic controls, but ultrastructural changes were demonstrable using morphometric techniques. In the group of 'poorly-controlled' rats the kidneys were increased in weight by 62%, the glomerular volume was increased by 32% [12], the basement membrane was increased in thickness by 6% [13], the mesangial region by 32% [15] and the basement membrane-like material by 30% [15]. In the group of 'well-controlled' diabetic rats these structures were not found to be different from the non-diabetic control group.

The above described changes may well represent the morphological lesions responsible for the abnormal albumin excretion; however, other factors, such as increased filtration pressure or decreased tubular reabsorption, could also be the cause of the abnormal albumin excretion in the diabetic rats in poor control.

Hostetter et al. [3] have shown changes in glomerular haemodynamics in Wistar-Munich rats after two months of insulin treated streptozotocin diabetes. However, in diabetic rats whose plasma glucose was very high these pronounced changes were not found. This could be due to the very severe diabetic state, which can inhibit some of the characteristic early changes in experimental diabetes. Seyer-Hansen has shown that the early increase in

kidney weight is correlated with blood glucose up to values of about 300 mg/ml [17], whereas this weight increase is absent in animals with severe hyperglycaemia resulting in loss of body weight [18].

In the human diabetic morphological as well as functional parameters have been extensively studied [4, 5, 9]. At the onset of diabetes patients in poor metabolic control have increased albumin excretion, GFR and kidney size [10], whereas glomerular basement membrane thickening is not present at the onset of juvenile diabetes [21]. Strict control of the diabetic state is able to reverse these abnormalities [10, 19]. A striking feature in human diabetes is the normal or slightly elevated urinary albumin excretion obtaining for many years in patients in the usual, only partly controlled metabolic state [9, 19] where we know that thickening of the glomerular basement membrane is developing.

The present investigations cannot disclose the pathophysiological mechanisms involved in the increased albumin excretion in streptozotocin diabetic rats in poor metabolic control. It could be due to the morphological abnormalities present and consequently be an early sign of the slowly progressing diabetic nephropathy. Increase in glomerular basement membrane thickness and increase in albumin excretion are present at the same time in these studies but no conclusion can be drawn as to the possible connection between the two phenomena. The other possibility, that the increased albumin excretion is due to functional changes alone also remains to be clarified. Improvement in glycaemic control has diminished or normalized albumin excretion in long-standing diabetes [7, 19] before morphological change could have occurred. Irrespective of the mechanisms involved, the study has shown that this abnormality can be prevented by good metabolic control of the diabetic state.

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