Kidney tissue insulin-like growth factor I and initial renal growth in diabetic rats: Relation to severity of diabetes

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Abstract The initial renal hypertrophy in experimental diabetes is dependent on the prevailing blood glucose level and is associated with renal accumulation of insulin-like growth factor I. To investigate the relationship of blood glucose to kidney IGF-I, a graded range of diabetic aberration was established in young rats by iv injection of increasing amounts of streptozotocin (25-80 mg/kg) at day 0. In 30 diabetic rats the mean of day 1 and day 2 blood glucose concentrations ranged from 6.2 to 32.0 mmol/l and 24-h urinary glucose excretion (24-48 h) from 0.04 to 43.3 mmol/24 h. The right kidneys were removed after 48 h, weighed and their IGF-I concentration analysed by radioimmunoassay. Kidney IGF-I was positively correlated to blood glucose \( r = 0.66, p<0.0001 \) as well as to 24-h urinary glucose output \( r = 0.54, p=0.005 \). At this early stage, kidney weight already correlated to blood glucose \( r = 0.60, p<0.0005 \). No relationship between kidney IGF-I and kidney weight was found. However, if animals with severe diabetes were excluded, a significant correlation could be established \( r = 0.51, p = 0.01, N = 24 \). The results support the hypothesis that IGF-I plays a causal role in the initial renal hypertrophy of experimental diabetes.

Induction of experimental diabetes causes a rapid renal enlargement accompanied by increases in total kidney protein and RNA demonstrable 48 h after the onset of glycosuria (1-3). This diabetic renal hypertrophy is preceded by a rise in kidney tissue insulin-like growth factor I (IGF-I) concentration, which reaches a peak 24-48 h after induction of diabetes (2,3). The kidney IGF-I accumulation pattern parallels that of labelled thymidine incorporation in renal tubule cells (4) being in good accordance with the mitogenic effects.

It has previously been demonstrated that the initial kidney hypertrophy in less severely diabetic animals, with blood glucose concentrations ranging from normal to about 19 mmol/l, is linearly dependent on blood glucose levels one week after induction of diabetes (5). On the other hand, if the metabolic derangement is too severe with blood glucose levels exceeding 25-30 mmol/l, the kidney growth is diminished or even abolished (1,6,7).

The main purpose of the present study was to investigate whether the degree of kidney IGF-I accumulation is also blood glucose dependent.

Materials and Methods

Male Wistar rats weighing between 246-291 g were made diabetic by iv injection of streptozotocin in acidic saline \( \text{pH} = 4.0 \). To induce different degrees of diabetes, STZ was given in amounts ranging from 25-80 mg/kg. Control rats received an equivalent volume of saline iv. Twenty-four hours after injection with STZ, the animals were placed in metabolic cages for 24-h collection of urine. Two days after STZ, the animals were anesthetized with sodium barbital (25 mg/kg) and the right kidneys were removed, trimmed of fat, hilus and capsule, weighed and immediately frozen in liquid nitrogen. Blood glucose was measured daily in tail blood (Haemo-Glucotest 1-44 and Reflolux II, Boehringer-Mannheim, Mannheim, FRG) and urine tested for ketone bodies with Neostix-4 (Ames, Stoke Poges, Slough, UK). The animals were weighed every day and had free access to standard fodder (Al-
tromin, Lage, FRG) and water throughout the experiment.

IGF-I extraction was performed according to D’Ercole et al. (8) as previously described (2,3). In brief, the frozen kidney was homogenized on ice in 1 mol/l acetic acid (3 ml/g tissue) with an Ultra Turrax TD 25 (Janke-Kunkel GmbH, Staufen, FRG) and further disrupted using a Potter Elvehjem homogenizer. The extract was placed on ice for 2 h, centrifuged at 4000 rpm for 15 min and the supernatant decanted. The pellet was re-extracted once, the supernatants pooled and lyophilized to dryness. The sample was redissolved in 40 mmol/l phosphate buffer, pH 8.0, in a ratio of 5 ml buffer per g tissue weight. Tissue extracts were kept at -20°C until IGF-I assay was performed in diluted extracts (1:40 and 1:80). IGF-I was estimated as previously described (2,3) using IGF-I antibody UB 286 (raised by L. E. Underwood and J. J. van Wyk, Pediatric Endocrinology, University of North Carolina, Chapel Hill, NC) donated by the National Hormone and Pituitary Program.

Fig. 1 illustrates the semilog linearity of biosynthetic IGF-I and the immunoreactivity of kidney extracts, indicating antigen similarity and that no binding proteins or receptors from the kidney extracts interfered in the radioimmunoassay. In addition, when biosynthetic IGF-I or an aliquot of the kidney extract was gel-filtered on Ultrogel(IBF) AcA 200, peaks in immunoreactive IGF-I occurred in the same tube fractions.

Urinary glucose content was measured by standard enzymatic technique (glucose oxidase method).

Relationships between parameters were analysed with linear regression and multiple regression analysis using the Statistical Package for the Social Sciences (SPSS inc., Chicago, USA). A value of 2p<0.05 was considered statistically significant.

Results

The saline-injected control rats had blood glucose concentrations below 5.5 mmol/l. Blood glucose values above 7.0 mmol/l at the time of killing were regarded as indicative of diabetes. The 30 diabetic rats had blood glucose concentrations ranging from 7.4 to 29 mmol/l at day 2 and none of the animals exhibited ketonuria. The glucose values used in regression analyses are the means of day 1 and day 2 blood glucose concentrations. The diabetic animals had urinary glucose outputs ranging from 0.04 to 43.3 mmol/24 h.

Fig. 2 A, B shows the blood glucose and 24-h urinary glucose excretions plotted against kidney IGF-I expressed in ng/g kidney tissue.

Including all animals, a positive correlation with kidney IGF-I concentration was found both for blood glucose (A) (r = 0.66, p<0.0001) and urinary glucose (B) (r = 0.54, p<0.005). When the kidney weights were plotted against IGF-I, no significant correlation could be established, but if animals with blood glucose concentrations exceeding 25 mmol/l were excluded, a significant correlation was found (r = 0.51, p = 0.01) (Fig. 3 A). In contrast, the exclusion of animals with severe diabetes did not influence the strength or significance of correlations between kidney IGF-I and the metabolic parameters.

In Fig. 3 B the correlation between blood glucose and kidney weight is shown (r = 0.60, p<0.0005); when excluding animals with blood glucose >25 mmol/l, the correlation tended to be stronger (r = 0.69, p<0.0001), but this difference did not reach significance (0.05<p<0.10). These results and the linear regression equations are shown in Table 1. In multiple regression analysis with kidney IGF-I concentration as the dependent variable, the overall r value was 0.72 (p<0.001) with only blood glucose remaining independently significant (partial standardized r = 0.54, p = 0.02).
Fig. 2.
Relations between A. blood glucose and kidney IGF-I (r=0.66, p<0.0001) and B. 24-h urinary glucose excretion and kidney IGF-I (r=0.54, p<0.005). A and B represent all animals (N=32).

Fig. 3.
Relations between A. kidney IGF-I and kidney weight (r=0.51, p=0.01) and B. blood glucose and kidney weight (r=0.60, p<0.0005). B represent all animals (N=32) whereas A consists of values only from diabetic animals with blood glucose concentrations below 25 mmol/l (N=24).
Table 1.
Results of linear regression analysis: blood glucose against kidney IGF-I and kidney weight; urinary glucose excretion against kidney IGF-I, and kidney IGF-I against kidney weight. *Includes only diabetic animals with blood glucose concentrations below 25 mmol/l.

<table>
<thead>
<tr>
<th>X</th>
<th>Y</th>
<th>Equation</th>
<th>N</th>
<th>r</th>
<th>2p</th>
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<tr>
<td>Blood glucose (mmol/l)</td>
<td>Kidney IGF-I (ng/g kidney)</td>
<td>( y = 6.8x + 187 )</td>
<td>32</td>
<td>0.66</td>
<td>&lt;0.0001</td>
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<td>-</td>
<td>Kidney weight (mg)</td>
<td>( y = 5.8x + 850 )</td>
<td>32</td>
<td>0.60</td>
<td>&lt;0.0005</td>
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<tr>
<td>-</td>
<td>Kidney weight (mg)</td>
<td>( y = 8.0x + 822 )</td>
<td>24*</td>
<td>0.69</td>
<td>&lt;0.0001</td>
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<tr>
<td>Urinary glucose (mmol/24 h)</td>
<td>Kidney IGF-I (ng/g kidney)</td>
<td>( y = 3.6x + 264 )</td>
<td>32</td>
<td>0.54</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Kidney IGF-I (ng/g kidney)</td>
<td>Kidney weight (mg)</td>
<td>( y = 0.2x + 889 )</td>
<td>32</td>
<td>0.23</td>
<td>NS</td>
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<tr>
<td>-</td>
<td>Kidney weight (mg)</td>
<td>( y = 0.6x + 794 )</td>
<td>24*</td>
<td>0.51</td>
<td>0.01</td>
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Discussion

It is now well established that induction of experimental diabetes causes a rapid increase in kidney IGF-I concentration preceding the increase in renal size (2,3). The kidney IGF-I accumulation is maximal between 24 and 48 h after STZ injection (2,3) and then returns after about four days to the basal level in good accordance with the rate of labelled thymidine incorporation into kidney tubular cells (4).

In the present study comprising diabetic rats with blood glucose ranging from very slightly elevated levels to values around 30 mmol/l, a positive correlation was found between blood glucose and kidney IGF-I concentration 48 h after induction of STZ diabetes. It has previously been shown that the degree of renal hypertrophy, after one week of mild to moderate diabetes in rats, is positively correlated to blood glucose (5). On the other hand if the metabolic derangement is too severe, with blood glucose levels exceeding 25-30 mmol/l, the kidney growth is diminished or even abolished (1,6). This is probably due to severe catabolism seen in these conditions, since an inversely related association is found between kidney weight and body weight 20 days after induction of experimental diabetes (1,7).

In the present experiment a positive correlation was found between blood glucose and kidney weight 48 h after induction of diabetes, even when including animals with blood glucose values around 30 mmol/l. The seeming discrepancy from the observations referred to above (1,5-7) may be due to the shorter diabetes duration, with catabolism having been too shortlived markedly to reduce the capacity for early kidney growth in the rats with very high blood glucose levels. Still, the correlation tended to be somewhat stronger when animals with hyperglycemia above 25 mmol/l were excluded. In contrast, the highly significant correlations between blood glucose or urinary glucose excretion and kidney tissue IGF-I concentration were unaltered if results from the severely diabetic animals were included, indicating that the degree of accumulation of this putative causal growth factor relies solely on the degree of hyperglycemia.

Furthermore, a significant relationship between kidney IGF-I and kidney weight was found, but only when results from animals with blood glucose values exceeding 25 mmol/l were excluded.

The relatively weak correlation between kidney IGF-I and kidney weight may indicate that the kidney cannot respond fully to the growth stimulus when the metabolic aberration is too severe. It appears, however, more likely that different time course changes of kidney IGF-I and kidney growth may account for the weak association, since the rise in kidney IGF-I concentration is maximal before the first demonstrable kidney growth.

We have previously demonstrated that normalization of blood glucose concentration by strict insulin treatment to diabetic rats prevents the in-
crease in both kidney IGF-I and renal hypertrophy (2). The observation that the degree of kidney IGF-I accumulation is directly proportional to the prevailing blood glucose levels in rats with graded severities of diabetes adds weight to the hypothesis that the renal accumulation of IGF-I is promoted by the diabetic state and that the rise in renal IGF-I may be a prerequisite for the degree of initial renal growth.

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References


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