Original Article

Role of insulin and the IGF system in renal hypertrophy in diabetic Psammomys obesus (sand rat)

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Abstract

Background. Diabetic nephropathy is caused by multiple factors related to the altered metabolic environment in diabetes mellitus (DM). Experimental diabetic kidney disease is characterized by renal hypertrophy associated with increased tissue concentrations of insulin-like growth factor I (IGF-I). To assess the specific roles of serum insulin and glucose in mediating the development of diabetic nephropathy, the effects of both hyperinsulinaemic and hypoinsulinaemic DM were studied in Psammomys obesus (sand rat), a model of type 2 DM.

Methods. The IGF-I system was studied in normal Psammomys obesus gerbils and at 5, 15 and 70 days after the induction of either hyper- or hypoinsulinaemic DM. To induce hyperinsulinaemic DM, Psammomys were raised on a high-energy diet. Hypoinsulinaemic DM was induced by either administration of streptozotocin or a specially designed diet.

Results. Hyperinsulinaemic hyperglycaemic Psammomys did not exhibit renal hypertrophy (unchanged kidney/body-weight ratio) and renal IGF-I levels were in the normal range on days 5, 15 and 70. In contrast, Psammomys with hypoinsulinaemic hyperglycaemia induced either by streptozotocin injection or by pancreas exhaustion brought on by a long-term caloric excess diet, had significant increases in kidney/body-weight ratio which were associated with elevated renal IGF-I and mRNA and protein levels of kidney IGFBP binding protein I.

Conclusions. This study shows that serum insulin levels in the presence of hyperglycaemia have an important role in the development of experimental diabetic nephropathy in the Psammomys model. The implication of this finding is that the pathophysiological mechanisms for diabetic kidney disease in experimental models may be different for type 1 and type 2 DM.

Keywords: diabetic kidney disease; insulin; insulin-like growth factor binding proteins; insulin-like growth factor I; Psammomys obesus

Introduction

Diabetic nephropathy is a major complication of both type 1 and type 2 diabetes mellitus (DM). The pathogenesis of diabetic kidney disease has not been fully determined, but there is strong evidence that the growth hormone (GH)insulin-like growth factor I (IGF-I) axis plays a major role in the early changes of diabetic kidney disease [1]. Rats given streptozotocin (STZ) develop renal hypertrophy, increased glomerular volume and hyperfiltration shortly after the onset of type 1-like diabetes [2]. These changes are associated with increases in renal IGF-I levels [3]. At the same time, protein and mRNA levels of various IGF binding proteins (IGFBPs) also change in response to the diabetic environment. The primary role of GH in this process is suggested by experiments in which GH-deficient diabetic animals have only mild renal hypertrophy as compared with their GH-replete littermates [1].

The specific roles of insulin and glucose in mediating the increases of IGF-I and IGFBPs in the kidney remain to be elucidated. Experimental diabetic animals treated with insulin have a reversal of renal hypertrophy [4]. At the same time, the changes occurring in the renal IGF-I system are also reversed. However, it is still unclear whether the effect of insulin treatment is due to a lowering of serum glucose or to an increase in insulin levels. If low insulin is the cause of increased IGF-I and altered IGFBP levels, then it is possible that
in cases of hyperinsulinism or insulin resistance (e.g. type 2 DM) the pathological factors contributing to diabetic kidney disease may be different from those of type 1 DM.

To determine the specific roles of insulin and glucose in inducing experimental diabetic kidney disease, we have studied the effect of hyper- and hypoinsulinemic DM on the development of kidney disease in Psammomys obesus (sand rat). Psammomys develops hyperinsulinism and hyperglycaemia on a high-energy diet, and has thus been used as a model of diet-induced type 2 DM [5,6]. In the present study, the effects of hyperinsulinemic hyperglycaemia on renal hypertrophy was compared with the hypoinsulinemic hyperglycaemia induced in the same animal by STZ or due to pancreatic insufficiency after long-term ingestion of a high-energy diet that causes pancreatic β-cell exhaustion and death. Our results indicate that there are significant differences in both renal hypertrophy and the IGF-I system in Psammomys with hypoinsulinemic diabetes as compared to either controls or hyperinsulinemic diabetic animals.

Subjects and methods

Animals and experimental protocol

Male Hebrew University Psammomys obesus gerbils were studied. The animals were maintained in an Animal Research Facility and the experiments performed according to the regulations of the Hadassah University Committee on Animal Care and Use. Psammomys were housed in a room with 12-h light on/light off cycle at 21°C and humidity of 55%. The animals had free access to food and tap water unless otherwise specified below.

Psammomys of the diabetic prone (DP) line of the Hebrew University Colony (Harlan, Jerusalem, Israel), were raised for approximately 40 days after weaning on a low-energy diet (LE group) consisting of carbohydrate 70%, fat 3.1%, protein 16.7%, and ash 10.2% in the form of hard-pressed pellets of total digestible energy of 2.4 cal/g. On this diet, Psammomys maintain a non-fasting glucose in the range of 4–6 mmol/l. To induce hyperinsulinemic hyperglycaemia, a group of the animals were transferred to a high-energy (HE) diet which is a high-energy diet that causes pancreatic insufficiency after long-term ingestion of the kidney tissue as described previously [3].

Kidney IGF-I extraction and IGF-I radioimmunoassay

IGF-I extraction was performed according to the method of D’Ercole as described previously [8]. Briefly, kidneys were homogenized in 1 ml of acetic acid with an Ultra Turrax TD25 and further disrupted using a Potter Elvehjelm homogenizer. The tissues were extracted twice. The sample was lyophilized and subsequently re-dissolved in a phosphate buffer, 40 mmol/l, pH 8.0. Tissue extracts were kept at -20°C until the IGF-I assay was performed in diluted extracts. A linear relationship was found between biosynthetic IGF-I and IGF-I immunoreactivity in the kidney at different concentrations. The measured kidney IGF-I concentrations were all corrected for serum IGF-I entrapped in the kidney tissue as described previously [3].

Western ligand blotting (WLB) for IGFBPs in kidney

SDS-PAGE and WLB to measure IGFBPs was performed as previously described [9]. Briefly, 200 µg of renal protein was electrophoresed in SDS-PAGE (10% polyacrylamide) in non-reducing conditions. The separated proteins were transferred by electro-elution onto nitrocellulose paper (Schleicher and Schuell, Munich, Germany), and then incubated with [125I]-IGF-I. Membranes were washed with TBS and the nitrocellulose sheets were autoradiographed with Kodak X-AR film. Specificity of the IGFBP bands was ensured by competitive co-incubation with unlabelled IGF-I (Bachem, Bubendorf, Switzerland). WBs were quantified by densitometry using a Shimadzu CS-9001 PC dual-wavelength flying spot scanner.

Northern blot analysis for IGFBP-1 in kidney

Preparation of RNA from kidney and northern blot analysis was performed as previously described [10]. Total RNA (20 µg) was electrophoresed and then transferred to a nylon membrane (Magnagraph, MSI, Westboro, MA). An IGFBP-1 cDNA probe was labelled by random priming (Boehringer Mannheim GmbH, Germany) and hybridized with the membrane. After washing, the membranes were exposed to Kodak X-AR film. The results were quantified using a
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Statistical analysis

Data given in the text are mean ± SEM. Statistical significance of changes between the different groups was carried out by either one-way analysis of variance (ANOVA) or unpaired two-tailed Student’s *t*-test. Statistical significance was defined as *P* < 0.05.

Results

*Psammomys* given a diabetogenic high-energy diet (HE group) developed hyperglycaemia within 24–48 h. Blood glucose remained elevated for up to 70 days (Table 1). Fasting plasma insulin levels gradually increased in the HE group and there were significant differences at 5, 15 and 70 days compared with animals on the low-energy diet (LE group, Table 1). Non-fasting insulin levels in diabetic *Psammomys obesus* are markedly higher (65 ± 4 and 336 ± 33 in the LE and HE groups, respectively) [5,6]. A 44% elevation in body weight was observed at 70 days in the HE group over that of LE control group (Table 2). The 70-day HE animals demonstrated that the *Psammomys* diabetic phenotype was characterized by hyperglycaemia, hyperinsulinism and increase in weight [5,7]. The hyperinsulinaemic, hyperglycaemic HE group had a markedly larger kidney weight at 5, 15 and 70 days compared to either the LE group or the HE group (Table 2). The increase in kidney size was not unexpected, as there was an increase in overall weight (Table 2) and the organ weight of most tissues including liver, heart and fat (data not shown) that is characteristic of the diabesity syndrome. A more important indicator of renal hypertrophy, kidney/body-weight ratio, remained unchanged in the hyperinsulinaemic HE *Psammomys* group for the duration of the study (Table 2). In addition, the HE *Psammomys* animal groups had no elevation in urinary microalbumin [1.5 ± 0.2 vs 1.4 ± 0.3 (10⁻³ g/24 h) after 5 days, 2.0 ± 0.2 vs 1.9 ± 0.3 (10⁻³ g/24 h) after 15 days, and 2.5 ± 1.0 vs 2.0 ± 0.5 (10⁻³ g/24 h) after 70 days in the HE and LE groups, respectively]. Furthermore, gross histological analysis of slices from kidney cortex demonstrated no signs of renal damage (data not shown).

In a second series of experiments, relative hyperinsulinaemic DM was induced in *Psammomys* animals either by injection of STZ or by maintenance on WEI diet, which results in relative hyperinsulinaemia due to diminished β-cell mass. These animals have lower plasma insulin levels compared to the HE group, and the glucose/insulin ratio even in the fasting state is markedly higher as compared to either the LE group or the HE group (Table 1).

An increase in kidney size and kidney/body-weight ratio are markers for early diabetic kidney disease. Kidney weight increased in both relative hypoinsulinaemic groups and in the HE group after 70 days of diabetes, compared with the control LE group (Table 2). In contrast to the HE group, the kidney weight/body-weight ratio increased significantly by 31%

### Table 1. Biochemical parameters of the control and diabetic animals

<table>
<thead>
<tr>
<th>Duration of diabetes</th>
<th>Treatment groups</th>
<th>n</th>
<th>Glucose (mmol/l)</th>
<th>Insulin (mU/l)</th>
<th>Glucose/insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 days</td>
<td>Control (LE)</td>
<td>8</td>
<td>3.9 ± 0.2</td>
<td>39 ± 13</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Diabetic (HE)</td>
<td>8</td>
<td>17.0 ± 1.2*</td>
<td>68 ± 11*</td>
<td>4.5*</td>
</tr>
<tr>
<td>15 days</td>
<td>Control (LE)</td>
<td>14</td>
<td>4.5 ± 0.3</td>
<td>45 ± 8</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>Diabetic (HE)</td>
<td>11</td>
<td>18.6 ± 2.7*</td>
<td>101 ± 13*</td>
<td>3.3*</td>
</tr>
<tr>
<td>70 days</td>
<td>Control (LE)</td>
<td>6</td>
<td>5.1 ± 0.4</td>
<td>55 ± 14</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>Diabetic (HE)</td>
<td>7</td>
<td>16.3 ± 2.0*</td>
<td>220 ± 24*</td>
<td>1.3</td>
</tr>
<tr>
<td>7 days</td>
<td>Diabetic (WEI)</td>
<td>6</td>
<td>26.9 ± 0.6b</td>
<td>48 ± 9</td>
<td>10.1*</td>
</tr>
<tr>
<td></td>
<td>Diabetic (STZ)</td>
<td>5</td>
<td>25.8 ± 2.4b</td>
<td>54 ± 23</td>
<td>8.6*</td>
</tr>
</tbody>
</table>

*P* < 0.05 compared with the control LE group; *P* < 0.01 compared with the control LE group and diabetic HE group at 70 days diabetes duration; *P* < 0.01 compared with LE, WEI and STZ groups.

### Table 2. Physical parameters of the control and diabetic animals

<table>
<thead>
<tr>
<th>Duration of diabetes</th>
<th>Treatment groups</th>
<th>n</th>
<th>Body-weight (g)</th>
<th>Kidney weight (g)</th>
<th>Kidney weight/body weight × 1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 days</td>
<td>Control (LE)</td>
<td>8</td>
<td>122 ± 4</td>
<td>1.86 ± 0.16</td>
<td>15.2 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Diabetic (HE)</td>
<td>8</td>
<td>131 ± 6</td>
<td>2.09 ± 0.30a</td>
<td>15.8 ± 0.4</td>
</tr>
<tr>
<td>15 days</td>
<td>Control (LE)</td>
<td>14</td>
<td>161 ± 6</td>
<td>2.15 ± 0.34</td>
<td>13.3 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Diabetic (HE)</td>
<td>11</td>
<td>169 ± 4</td>
<td>2.41 ± 0.28a</td>
<td>14.2 ± 0.4</td>
</tr>
<tr>
<td>70 days</td>
<td>Control (LE)</td>
<td>6</td>
<td>181 ± 6</td>
<td>1.79 ± 0.25</td>
<td>9.8 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Diabetic (HE)</td>
<td>7</td>
<td>261 ± 5a</td>
<td>2.71 ± 0.26b</td>
<td>10.3 ± 0.3</td>
</tr>
<tr>
<td>7 days</td>
<td>Diabetic (WEI)</td>
<td>6</td>
<td>208 ± 9ae</td>
<td>2.81 ± 0.27b</td>
<td>13.5 ± 0.5c</td>
</tr>
<tr>
<td></td>
<td>Diabetic (STZ)</td>
<td>5</td>
<td>152 ± 6ad</td>
<td>2.35 ± 0.14bd</td>
<td>15.5 ± 0.3c</td>
</tr>
</tbody>
</table>

*P* < 0.05 compared with the control LE group at the same age; *P* < 0.001 compared with the control LE group at the same age; *P* < 0.001 compared with the LE and HE groups at 70 days diabetes duration; *P* < 0.05 compared with the WEI group.
and 50% in both relative hypoinsulinaemic WEI and STZ groups, respectively (Table 2).

Renal IGF-I levels have been strongly associated with renal hypertrophy [3]. In Psammomys animals, the kidney IGF-I levels increased significantly after 7 days of diabetes by 34 and 37% in the hypoinsulinaemic WEI and STZ animals, respectively, whereas there were no significant changes in the kidney IGF-I levels after 5, 15 and 70 days of diabetes in the hyperinsulinaemic HE Psammomys (Figure 1). Serum growth hormone levels were unchanged in either the hyperinsulinaemic (19 ± 7 mg/ml) or the hypoinsulinaemic animals (23 ± 4 and 20 ± 5 mg/ml in the STZ and WEI groups, respectively) compared to the LE group (17 ± 4 mg/ml).

In previous studies we have shown that renal 30-kDa IGFBP fraction that contains mainly IGFBP-1 protein is consistently increased in STZ-induced diabetes in rats [10]. Relative hypoinsulinaemic diabetes, in either the STZ or the WEI Psammomys groups, was associated with a significant elevation in the renal 30-kDa IGFBP fraction. In contrast, animals with hyperinsulinaemic diabetes did not show any significant change in the renal 30-kDa IGFBP fraction (Figure 2). The increase in IGFBP-1 in other experimental models has been attributed to increased levels of IGFBP-1 mRNA. As shown in Figure 3, there is a significant 100 and 75% increase in kidney IGFBP-1 mRNA in hypoinsulinaemic WEI and STZ Psammomys groups compared with either the control LE group or the hyperinsulinaemic HE group. As different from previous description in STZ diabetic rats, Psammomys kidney IGFBP-3 protein levels were significantly higher in the HE (70 days diabetes duration), WEI and STZ diabetic Psammomys groups (Figure 4).

Discussion

In earlier studies, we and others [2,9,10] have shown that in the STZ diabetic rat, there is an increase in renal size and glomerular hypertrophy that begins in the earliest phases of diabetes and is associated with an increase in renal IGF-I. There is strong evidence to suggest that the increase in IGF-I causes the renal enlargement, although other mediators have been implicated in the causation of diabetic kidney disease, including growth factors (e.g. transforming growth factor β, vascular endothelial growth factor, nitric...
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oxide), altered renal haemodynamics, advanced glycosylation end-products, oxidative stress and abnormalities in blood coagulation [11–15]. Also, in studies of experimental type 2 diabetes, there is considerable heterogeneity in the nature of diabetic kidney disease among animal models [16,17].

Regarding pathogenesis, it is not clear whether insulin or glucose levels promote the increase in renal IGF-I, as it has been shown that both high glucose and reduced insulin levels serve as triggers for specific transduction pathways. In order to address this issue, we studied both hyperinsulinaemic and hypoinsulinaemic DM in the same animal model, the diabetes prone mouse *Psammomys obesus*. Our studies indicate that insulin plays a major role in the pathogenesis of experimental nephropathy in the *Psammomys*.

In these animals, DM associated with hyperinsulinaemia is not associated with the pathological changes seen in hypoinsulinaemic DM. Insulin levels appear to be related to changes in the GH/IGF-I system as levels of renal IGF-I increased in the presence of relatively low insulin levels, as indicated by the high glucose/insulin ratio. However, renal IGF-I did not increase when the levels of insulin were markedly elevated, as is typical for type 2 DM in *Psammomys*. In addition, changes in IGFBP expression characteristic of experimental hyperinsulinaemia in rats with STZ-induced DM were not altered in the hyperinsulinaemic *Psammomys*.

Prolonged dietary excess in *Psammomys* is associated with a transition from hyperinsulinaemic to hypoinsulinaemic DM as a result of enhanced β-cell apoptosis [7], and the consequent lack of insulin may be responsible for the morphological changes seen in the late course of DM in the *Psammomys*. However, fasting insulin levels in WEI and STZ *Psammomys* gerbils decreased significantly, but not to levels lower than in control animals, and the glucose/insulin ratio remained markedly higher than in the LE and HE groups. It seems that severe hypoinsulinaemia in this condition is prevented partly by *Psammomys* secreting large amount of pro-insulin (comprising more than one-half of the immunoassayable insulin) under the stress of severe hyperglycaemia [18]. As is well known, immunoassayable pro-insulin has a negligible biological effect, which may explain the marked hyperglycaemia and the kidney response in these animals.

It should be noted that although hyperinsulinaemic diabetic *Psammomys* do not exhibit changes similar to those of the STZ- or WEI-induced diabetic *Psammomys*, they do develop significant diabetic kidney disease. This was documented by Marquie *et al.* [19], who showed that *Psammomys* developed significant mesangial proliferation and capillary basement membrane thickening after 12–15 months of hyperinsulinaemic diabetes. Thus it is probable that diabetic kidney disease develops more slowly in the animal model of type 2 than in the hypoinsulinemic animal model of type 1 diabetes. The signs of early kidney changes in diabetes (i.e. renal hypertrophy and increased renal IGF-I levels) that occur within days of the development of type 1 DM are delayed in hyperinsulinaemic animal models of type 2 DM. However, renal growth was shown to be stimulated in an animal model of type 2 DM by glucose-dependent pathways even in the presence of normal levels of IGF-I [16].

Our study suggests that insulin is a key mediator in renal hypertrophy and in the increased renal IGF-I in *Psammomys*, a model of type 2 DM. This is supported by the findings of Kaufman and Catanese [20] who demonstrated that STZ-induced diabetic rats in a normoglycaemic state induced by phlorizin treatment, had elevated renal IGF-I levels, as opposed to animals treated with insulin, indicating that low insulin plays a key role in the induction of renal IGF-I overexpression. In other experiments from our laboratory, hyperglycaemia with relative normoinsulinaemia induced in rats by low-dose STZ resulted in a delay in the increase in renal IGF-I and renal hypertrophy as compared to animals injected with high-dose STZ.

IGFBPs have been extensively studied in the diabetic state. In the kidney of animal models for type 1 DM, IGFBP-1 levels increase in the renal cortex in both the early and late stages of diabetes. It has been suggested that IGFBP-1 mediates the effects of IGF-I on kidney cells by enhancing delivery of IGF-I to the cell surface. The increase in the level of this binding protein may be important in the development of diabetic kidney disease [1]. In the present study there was no change in IGFBP-1 mRNA or protein in the hyperinsulinaemic hyperglycaemic animals. These findings support the observation of Kaufman and Catanese [20], who showed that IGFBP-1 mRNA increases in response to hypoinsulinaemia rather than hyperglycaemia.

In conclusion, we have shown that hypoinsulinaemia is associated with renal hypertrophy in an animal model of type 2 DM, while hyperinsulinaemia protects the kidney from developing typical metabolic and structural changes characteristic of type 1 DM. Further studies are necessary to clarify the causal relationship between insulin and glucose levels and the development of kidney disease.

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**References**


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