Effect of Chronic Growth Hormone Administration on Diabetic Nephropathy in the Rat*

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ABSTRACT

Indirect data exist which implicate elevated growth hormone (GH) as a factor in the development of diabetic nephropathy. The administration of somatostatin (SRIH) has been shown to reverse many of the changes found in early diabetic nephropathy; however, it is unknown whether SRIH causes these effects by the suppression of GH or by other unspecified factors. To study directly the possible effect of excess GH in the development of diabetic nephropathy, either ovine growth hormone (0.2 mg oGH) or diluent buffer was administered IM daily for 19 weeks to diabetic rats and to controls. Severity of nephropathy was assessed by 24 hour urine albumin excretion (UAE), relative kidney weight, and kidney histology. Results showed that diabetic rats overall had elevated UAE and kidney weight vs non-diabetic rats (46.2 ± 8.6 vs 5.4 ± 1.3 mg per day and 5.7 ± 0.2 vs 2.7 ± 0.1 mg per g of body weight, respectively, p < 0.001). However, no differences were detected between diabetic rats treated with GH compared to control diabetic rats. Additionally, diabetic rats had histopathologic changes consistent with early diabetic nephropathy, but no difference in severity scores was found between diabetic groups. These data provide evidence against GH as an etiologic factor in the development of diabetic nephropathy and it is speculated by the authors that SRIH exerts its protective renal effects in diabetes by mechanisms other than GH suppression.

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EFFECT OF CHRONIC GROWTH HORMONE ADMINISTRATION ON DIABETIC NEPHROPATHY 463

Introduction

Diabetic nephropathy is one of the most important causes of long term morbidity and mortality in patients with insulin-dependent diabetes mellitus (IDDM) and remains the leading cause of chronic renal insufficiency in the United States today. Despite these facts, the specific etiologic factors underlying the functional and structural changes in the kidney in patients with diabetic nephropathy are still debated. Levels of growth hormone (GH) are known to be elevated in patients with IDDM, and studies exist which indirectly implicate GH and the GH dependent growth factor, insulin-like growth factor 1 (IGF-1), as possible factors in the development and progression of nephropathy in diabetes mellitus. Both somatostatin (SRIH) and its long-acting analogue, octreotide, have recently been reported in both human and animal studies to be effective in normalizing many of the early changes of diabetic nephropathy, including kidney hypertrophy, elevation of glomerular filtration rate (GFR), and increased urinary albumin excretion (UAE). It is unknown whether SRIH exerts its protective effects on the diabetic kidney by suppression of GH or by other as yet unspecified renal effects.

In order to study directly the possible role of GH excess in the development of diabetic nephropathy, high doses of GH were administered chronically to rats with experimental diabetes and to controls. After 19 weeks, severity of nephropathic changes was assessed by UAE and histologic analysis.

Methods

Experiments were conducted using male Sprague-Dawley rats, approximately 150 grams.* Animals were maintained on tap water and standard rat chow (Purina) ad libitum. Diabetes was induced by the injection of streptozocin, † 50 mg per kg in 0.1 M sodium citrate buffer, pH 4.5, IV, after a 12 hour fast. Control animals were injected with buffer alone. Diabetes was confirmed by semi-quantitative measurement of urine glucose. Both diabetic and non-diabetic groups were randomly divided to receive either oGH, 0.2 mg IM in 0.15 M NaCl, 0.03 M NaHCO₃ buffer, pH 9.0 or buffer alone. Injections were continued daily for 19 weeks at which time the animals were sacrificed. This length of treatment was chosen to allow sufficient time for pathologic kidney changes and increased albuminuria to develop but to conclude the experiment before end stage changes ensued so as to allow for the detection of potential differences between treatment groups. Glycosylated hemoglobin was measured by affinity chromatography columns at nine weeks and at 19 weeks‡ to monitor the severity of hyperglycemia in each group.

Presence and severity of diabetic nephropathy were assessed by several parameters at the end of 19 weeks. Urine output over 24 hours was collected using standard metabolic cages, and UAE was measured using a commercially available enzyme-linked immunosorbent assay system§ which has an assay sensitivity of 1.5 mcg per ml. All samples were assayed in duplicate, and the average intraassay variation was 5.2 percent. Urine samples were stored at −20°C until assayed.

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† The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.
After euthanasia, the right kidney was removed and weighed. The left kidney was sectioned and prepared for both electron and light microscopy. Specifically, for light microscopy, the tissue was fixed in 10 percent buffered formalin, and sections were stained with hematoxylin and eosin, Periodic acid Schiff, and Masson's trichrome. For electron microscopy (EM), tissue was fixed in five percent buffered gluteraldehyde, rinsed in Sorensen's phosphate buffer and post-fixed in one percent osmium tetroxide. It was then dehydrated in graded alcohol and cleared with propylene oxide before epoxy resin embedding. Thick sections of 0.5 to 1.0 micron were stained with 0.1 percent methylene blue for orientation. Ultra-thin sections were stained with uranyl acetate and lead citrate. Micrographs were taken using a Zeiss-1091 electron microscope.

By light microscopy, the glomeruli were evaluated for an increase in endocapillary cells, polymorphonuclear nuclear (PMN) infiltrate, increase in mesangial matrix, and thickening of peripheral capillary walls as well as for the presence of sclerotic lesions. The tubules were examined for atrophy, dilatation, or necrosis. By EM, the glomeruli were graded for mesangial cellular and matrix expansion, capillary and basement membrane thickening, effacement of foot processes, and immune complex deposition. These parameters were evaluated in a blinded manner separately by two nephropathologists (SGS and TTA) using a semi-quantitative severity score, graded as 0 (no change), 1 (mild changes), 2 (moderate changes), or 3 (severe changes).

Results of UAE are expressed as mg per day, and kidney weight is expressed as mg per g of body weight. All values are mean ± SEM. Statistical significance of group differences was assessed by two-way analysis of variance (ANOVA) using the SPSS software package. In analysis of histologic data, ordinal data were assumed, and scores were analyzed using both ANOVA and Pearson chi square tests.

All procedures and experiments were approved by the institution's animal use and clinical investigation committees.

Results

Values for percent glycosylated hemoglobin are given in table I. Significant elevations were noted in both diabetic groups (F = 469.4, df = 1, p < 0.001) at nine and at 19 weeks. However, there were no significant differences in glycosylated hemoglobin levels between diabetic groups at any time, indicating equivalent levels of hyperglycemia throughout the experiment in the diabetic and diabetic + GH groups. Weight change over the entire 19 weeks was significantly higher in control animals compared to diabetic rats (F = 251.67, df = 1, p < 0.001) and also significantly higher overall in animals who received GH (F = 4.24, df = 1, p < 0.05) (table II).

In table II are also shown the results for 24 hour UAE and for relative kidney weight (mg per g of body weight) after 19

<p>| TABLE I |
| Percent of Glycosylated Hemoglobin |</p>
<table>
<thead>
<tr>
<th>Group (n)</th>
<th>9 Weeks</th>
<th>19 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (5)</td>
<td>5.8 ± 0.2</td>
<td>7.1 ± 0.2</td>
</tr>
<tr>
<td>Control + GH (7)</td>
<td>6.4 ± 0.2</td>
<td>6.8 ± 0.1</td>
</tr>
<tr>
<td>Diabetic (10)</td>
<td>15.3 ± 0.3*</td>
<td>16.7 ± 0.5*</td>
</tr>
<tr>
<td>Diabetic + GH (10)</td>
<td>15.2 ± 0.4*</td>
<td>17.3 ± 0.5*</td>
</tr>
</tbody>
</table>

* p < 0.001 vs. control groups. GH = growth hormone
TABLE II
Weight Gain, Urine Albumin Excretion, and Relative Kidney Weight at 19 Weeks*

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Weight Gain (g)</th>
<th>Urine Albumin Excretion (mg/day)</th>
<th>Kidney Weight (mg/g body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>342 ± 17</td>
<td>3.4 ± 0.6</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td>Control + GH</td>
<td>7</td>
<td>372 ± 18</td>
<td>6.8 ± 2.1</td>
<td>2.6 ± 0.2</td>
</tr>
<tr>
<td>Diabetic</td>
<td>10</td>
<td>118 ± 14</td>
<td>40.7 ± 9.5</td>
<td>5.6 ± 0.6</td>
</tr>
<tr>
<td>Diabetic + GH</td>
<td>10</td>
<td>145 ± 8</td>
<td>51.7 ± 14.3</td>
<td>5.8 ± 1.3</td>
</tr>
</tbody>
</table>

*Mean ± SEM. GH = growth hormone.

a p < 0.05 vs. groups not receiving growth hormone.

b p < 0.001 vs. control groups.

weeks. Both diabetic groups demonstrated significant elevations in UAE (F = 13.5, df = 1, p < 0.001), consistent with changes found in early diabetic nephropathy, but no difference was noted between diabetic rats receiving GH compared to diabetic rats receiving buffer only. This same pattern of findings was noted when analyzing relative kidney weight, a reflection of overall kidney hypertrophy.

Analysis of data on glomerular histology yielded overall mean severity scores for diabetic and control animals, respectively, of 1.75 ± 0.10 vs 0.75 ± 0.18 (F = 27.7, df = 1, p < 0.001) for mesangial hypercellularity, and 1.80 ± 0.14 vs 1.25 ± 0.25 (F = 4.2, df = 1, p = 0.05) for mesangial matrix expansion, indicating that diabetic animals had developed significant histologic abnormalities over the 19 weeks of the experiment. However, once again, there was no significant difference in the severity scores of the above parameters between GH treated diabetic rats and diabetic rats not receiving GH (1.8 ± 0.13 vs 1.7 ± 0.14, and 1.6 ± 0.16 vs 2.0 ± 0.21 for mesangial hypercellularity and matrix expansion, respectively).

Similarly, when analyzing for presence of glomerulosclerotic lesions, there was a statistical trend towards a higher percent of kidneys studied with lesions present in the diabetic group compared to the control animals (55 percent vs 25 percent, p = 0.10 by chi square test) but again, no difference was seen between diabetic rats receiving GH and untreated diabetic rats (50 percent vs 60 percent, respectively). There were no differences in any of the other histologic criteria examined, including changes in proximal and distal tubules and glomerular basement membrane thickness, between diabetic and non-diabetic animals.

Discussion

The current study provides clear direct evidence against chronic GH excess as an etiologic factor in the development of diabetic nephropathy. Nineteen weeks of daily high dose exogenous administration of GH did not worsen the developing diabetic kidney disease as measured by several different functional and structural parameters. The dose and species of GH used did have biologic effects, however, as was demonstrated by the increased weight gain seen in the groups treated with GH. Other studies involving ovine GH in rodents also confirm that it is biologically active in these species.14,15

The role of GH excess in the development of diabetic microangiopathy is controversial. Multiple studies have documented elevated GH levels in
Several past reports exist which provide indirect evidence supporting the possibility that GH and/or the GH dependent peptide IGF-1 may be important in the etiology of diabetic kidney disease. Flyvbjerg, et al²⁸ have reported that the early kidney hypertrophy seen in experimental diabetes in rats is accompanied or preceded by an increase in intra-renal IGF-1 content. In addition, they and others⁶,²⁷ have also shown that administration of the SRH analogue octreotide blocks both the initial kidney hypertrophy, as well as the increase in intra-renal IGF-1 content,⁶ and that GH deficient rats show an attenuation in these same parameters when studied over seven days of STZ-induced diabetes.⁵ Contrary to this, however, the same authors have also recently reported that short-term high-dose continuous IGF-1 infusion in diabetic rats did not worsen the observed relative kidney hypertrophy (kidney weight/body weight).⁴

Both SRH and octreotide have been reported recently to be effective in lowering UAE, reversing hyperfiltration, and in blocking or reversing kidney hypertrophy in studies of both humans and animals with diabetes.⁷,¹²,²⁵,²⁹ Most of the speculation regarding the underlying mechanism of action of SRH in this setting has centered on its ability to suppress GH.¹⁶,²⁵ However, levels of GH are not universally suppressed in studies involving SRH or octreotide treatment despite beneficial clinical effects of drug therapy.⁷,²⁵ Older studies, initially by Lundbaek,¹¹ have reported that hypophysectomy is effective in treating diabetic microangiopathy. It is unclear, however, whether the benefits seen were specifically due to the lowering of GH levels or to other factors. In addition, patients with IDDM have been reported to develop nephropathy despite also having severe GH deficiency.²⁰

Scant data exist concerning the effect of the administration of GH on the development and progression of diabetic nephropathy. A brief report by Osterby, et al initially stated that chronic GH administration to STZ-diabetic rats worsened glomerular basement membrane thickening over sixteen to twenty weeks.¹⁷ However, in a later study, the same authors reported that they could not confirm their initial findings.²⁶ Other reports have shown that short-term GH infusion in humans will result in renal hyperfiltration, as is seen in IDDM patients.¹⁰,²²,²³ In related interesting reports, transgenic mice which overproduce bovine or mouse GH have been shown to develop glomerulosclerotic lesions resembling those seen in diabetes.¹,²,¹⁹ The current authors are unaware of any other reports studying the direct effects of chronic GH administration on kidney disease in experimental diabetes. In addition, none of the earlier studies of administration of GH to diabetic rats investigated the effects of GH on the development of microalbuminuria as has been done in the current study.

Several different mechanisms have been proposed in the past by which GH might effect the development or severity of diabetic nephropathy. As stated earlier, short-term GH infusion does lead to renal hyperperfusion and hyperfiltration, which is thought by many to be one of the earliest and most important changes observed in the diabetic kidney.¹³,²¹ However, when studied in patients with IDDM, there was no correlation between levels of GH and the degree of elevation of GFR.³⁰ It has also been suggested that the chronic GH excess seen in humans with IDDM may stimulate the formation of various growth factors at the cellular level, leading to changes in glomerular mesangial and endothelial cells as well as to overall kidney hypertrophy.³,²⁴ To date, this theory remains speculative, and the results of our current study argue against this scenario concerning the possible effects of GH in diabetic nephropathy.
In conclusion, the current data would seem to provide evidence against GH as an etiologic factor in the development of diabetic nephropathy. It is therefore speculated by us that SRIH and octreotide may exert their protective renal effects in diabetes by mechanisms other than GH suppression. Further study as to the actual mechanism of action of these drugs in the treatment of diabetic kidney disease may lead to more specific and effective methods to treat or prevent this serious complication of diabetes mellitus.

Acknowledgements

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