JB3, an IGF-I Receptor Antagonist, Inhibits Early Renal Growth in Diabetic and Uninephrectomized Rats

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Abstract. Biochemical evidence suggests that insulin-like growth factor I (IGF-I) may play an important role as a mediator of kidney growth. In the present study, an IGF-I receptor antagonist (JB3) was synthesized, and its effect on the renal growth that follows the induction of diabetes or unilateral nephrectomy (UNx) was examined. JB3 was generated by solid phase peptide synthesis. Its activity as an IGF-I antagonist was confirmed in an opossum kidney cell line from its inhibitory effect on the increase in thymidine incorporation into DNA induced by recombinant human IGF-I. Male Wistar rats were anesthetized with halothane and subjected to either the induction of diabetes by streptozotocin (intravenous 60 mg/kg) for 4 d (control animals received citrate buffer) or UNx for 11 d (control animals were sham operated). JB3 was delivered by subcutaneous infusion using an osmotic minipump implanted 3 d before the induction of diabetes or UNx. Kidney wet weight, DNA, and protein all were significantly higher 4 d after the induction of diabetes (24%) or 11 d after UNx (55%). Dose-response studies (1 to 30 μg/kg per day) showed JB3 administration to inhibit the increase in kidney growth in both diabetic and UNx rats. The increase in kidney wet weight, DNA, and protein was significantly lower in UNx rats that were treated with JB3 10 μg/kg per day (P < 0.05) than in saline vehicle controls but was abolished in diabetic rats that were treated with JB3 3 μg/kg per day (P < 0.01). Increasing the dose of JB3 to 30 μg/kg per day was associated with a decrease in its inhibitory effect, resulting in bell-shaped dose-response curves. JB3 administration had no effect on the blood glucose concentration or food consumption by either diabetic or nondiabetic animals. The results support the concept of IGF-I as an important mediator of the early renal growth that follows the induction of diabetes or UNx in the rat.

Insulin-like growth factor I (IGF-I) is a potent mitogen proposed to play an important role in the regulation of cell proliferation, apoptosis, and tumorigenicity (1). Kidney growth is enhanced after the systemic administration of exogenous recombinant human IGF-I to the rat (2), and glomerular hypertrophy is a feature of the overproduction of IGF-I in transgenic mice (3). The renal growth that follows either the induction of diabetes or unilateral nephrectomy (UNx) has previously been attributed to an increase in the kidney content of IGF-I protein (4), located principally in the collecting duct and ascending limb, suggested to precede the growth response (5,6).

Diabetes is associated with a sustained fall in hepatic IGF-I mRNA (7), and even though serum IGF-I declines (8), the systemic circulation has been proposed to be the source of IGF-I protein that accumulates in the kidney (9). Upregulation of kidney IGF-I receptor mRNA and receptor protein have also been observed (10,11), and opposing regional changes in insulin-like growth factor-binding protein I (IGFBP-I) and IGFBP-5 between the renal medulla and cortex have been described (12). Time course studies suggest the increase in renal IGF-I protein to be paralleled by an increase in renal IGFBP-1 rather than by an increase in the IGF receptor protein (12). In contrast, after UNx, the increased renal accumulation of IGF-I is not associated with a change in the serum IGF-I concentration (13), and both serum IGFBP (1–4) and kidney tissue IGFBP (1–4) were reported to remain unchanged (14). Convincing evidence for an increase in renal IGF-I mRNA and IGF-I receptor mRNA has been obtained in the immature rat only in the 48-h period after UNx (15), whereas 7 d after UNx in weanling rats, an increase in mRNA for IGFBP 3, 4, and 5 has recently been detected (16). In the adult rat, compensatory renal hypertrophy was suggested to be dependent on growth hormone (GH) (17). However, a role for GH as a mediator of compensatory renal growth is not supported by studies using the Lewis dwarf rat, which is GH deficient (18,19).

The precise role of IGF-I in kidney growth may be difficult to define solely from the complex pattern of changes in renal IGF-I biochemistry that follow the induction of diabetes or a reduction in renal mass. Slow progress in establishing a precise role for IGF-I in kidney growth has previously been attributed to a lack of available IGF-I receptor antagonists (20). However, the three-dimensional modeling of the human IGF-I protein has allowed the synthesis of selective antagonists of the IGF type 1 receptor designed to mimic the D-domain of the IGF-I.
protein (21). These selective antagonists include a series of 12 amino acid cyclic peptides, one of which, JB3, is synthesized from dextro-isomer rather than levo-isomer amino acids, making it less susceptible to proteolytic degradation. JB3 therefore may be used in vivo (22). In the present study, the effect of JB3 on kidney growth was examined in the rat after either the induction of diabetes by streptozotocin (STZ) or UNx.

**Materials and Methods**

Male Wistar rats (Sheffield University) were housed 2 to 3 per cage at an ambient temperature of 17°C, humidity of 45%, and light/dark cycles of 12 h. All rats were allowed free access to a standard laboratory chow (protein casein content, 18%) and tap water. All experimental procedures were carried out according to the rules and regulations laid down by the Home Office (Animal Scientific Procedures Act, UK, 1986).

**Drug Administration**

Three d before UNx or the induction of diabetes (defined as day 0), an osmotic minipump (Alzet 200 µl, Alza, Palo Alto, CA) was implanted into each rat subcutaneously under halothane anesthesia. A subcutaneous loading dose of 0.1 ml/100 g body wt was also administered at the time of pump implantation (22). All minipumps were primed for 4 h at 37°C in isotonic saline before use.

**STZ-Induced Diabetes**

Three d after pump implantation (Alzet 2001, 7-d pump), rats were anesthetized with halothane (day 0) and either STZ 60 mg/kg in citrate buffer (pH 4) or citrate buffer (pH 4) was administered into a caudal vein in a dose volume of 0.1 ml/100 g body wt. The presence of diabetes was confirmed from the measurement of blood glucose concentration. Four d after the induction of diabetes, kidneys were removed under halothane anesthesia, weighed, and stored in liquid nitrogen before tissue analysis.

An initial dose-response study (JB3 1 to 30 µg/kg per day) was performed in five groups of 12-wk-old diabetic rats (n = 6 per group) matched for body weight (group means ranged from 361 ± 5 g to 374 ± 29 g) together with a diabetic control group receiving the saline vehicle. Dose-response curves were constructed for the inhibitory effect of JB3 administration on the increase in kidney wet weight, DNA, and protein associated with UNx using mean values obtained from the left kidney (n = 6 per group) in the following formula:

\[
\text{[diabetic (JB3) − nondiabetic (saline)/diabetic (saline) − nondiabetic (saline)]} \times 100
\]

The blood glucose concentrations of each group (17.6 ± 2.1 to 22.8 ± 4.1 mmol/L) were elevated as compared with citrate buffer controls but did not differ significantly from one another. JB3 at 3 µg/kg per day, the dose that produced the largest inhibition of kidney wet weight, was then used in an additional study in which 12-wk-old Wistar rats were randomized into four experimental groups of equivalent mean body weight (n = 6 per group) and subjected to (1) citrate buffer + saline, (2) citrate buffer + JB3 (3 µg/kg per day), (3) STZ 60 mg/kg + saline, or (4) STZ 60 mg/kg + JB3 (3 µg/kg per day).

**Unilateral Nephrectomy**

Three d after pump implantation (Alzet 2002, 14 d), rats were anesthetized with halothane (day 0). The right kidney was exposed through a flank incision, the adrenal gland was separated from the upper pole, and the kidney was decapsulated. The renal pedicle was ligated, and the kidney was removed. Sham-operated rats underwent a similar flank incision. Eleven d after UNx, animals were killed under halothane anesthesia. Kidneys that were removed during nephrectomy or at death were weighed and stored in liquid nitrogen before tissue analysis.

An initial dose-response study (JB3 1 to 30 µg/kg per day) was performed in five groups of 8-wk-old UNx rats (n = 6 per group) matched for body weight and included a UNx control group receiving the saline vehicle. Dose-response curves were constructed for the inhibitory effect of JB3 administration on the increase in kidney wet weight, DNA, and protein associated with UNx using mean values obtained from the left kidney (n = 6 per group) in the following formula:

\[
\text{[UNx (JB3) − sham (saline)/UNx (saline) − sham (saline)]} \times 100
\]

JB3 at 10 µg/kg per day, the dose that produced the largest inhibition of kidney wet weight, was chosen for further study in which 8-wk-old rats were randomized into four experimental groups of equivalent mean body weight (n = 6 per group) and subjected to (1) sham operation + saline, (2) sham operation + JB3 (10 µg/kg per day), (3) UNx + saline, or (4) UNx + JB3 (10 µg/kg per day).

**Peptide Synthesis**

JB3 was synthesized from D-isomer amino acids by solid phase peptide synthesis in the sequence Cys-Ser-Lys-Ala-Pro-Lys-Leu-Pro-Ala-Ala-Tyr-Cys (Figure 1) and purified by high-pressure liquid chromatography (21,23). Cys-Cys cyclization was performed by oxidation, and the presence of cyclization (as opposed to dimerization) was confirmed by mass spectrometry.

**Cell Culture**

The efficacy of JB3 as an IGF-I receptor antagonist was evaluated in OK cells, a cell line with features of the proximal tubule epithelium, derived from opossum kidney. Cells were cultured in Dulbecco’s Modified Eagles Medium (Sigma, Poole, UK) containing L-glutamine, 5.0% fetal calf serum, 100 IU/ml penicillin, and 100 µg/ml streptomycin (Sigma). All cultures were maintained at 37°C and in a
humidified atmosphere of 5% CO₂ in air. Cells were plated in 75 cm² culture flasks and reached confluence within 48 h.

The fetal calf serum concentration in the medium was reduced to 0.5%. Recombinant human IGF-I (rhIGF-I; Kabi, Uppsala, Sweden) was added to the medium at a concentration of 50 ng/ml, and incubation continued for an additional 48 h, followed by the addition of 2 μCi of [³H] thymidine (Amersham, Pharmacia Biotech, Little Chalfont, UK) to each flask for an additional 5 h. JB3 was added to the incubation medium 3 h before the addition of rhIGF-I.

The medium was removed, and the cells were washed three times in the presence of fresh 4 mM thymidine (Sigma). Ten ml of 5% TCA was added to each flask, and the cells were incubated at 37°C for an additional 90 min. Finally, 4 ml of 0.5 M NaOH was added to each flask, and the cells were incubated with gentle rocking at 37°C for 2 h. A total of 400 μl of this volume was taken from each flask and added to 4 ml of scintillation fluid and counted for [³H].

Renal Growth

Kidneys were removed from rats under halothane anesthesia, immediately weighed (wet weight), sectioned, and stored in liquid nitrogen. Tissue aliquots were dried to constant weight, and the kidney dry weight was calculated. No change in total kidney water was obtained in either diabetic or UNx studies. After UNx, compensatory renal growth (CRG) was assessed by comparing the wet weight of the right kidney at nephrectomy with that of the left kidney at sacrifice in each animal. CRG was expressed in percentage terms as the difference in weight between the left and right kidney as a proportion of the weight of the right kidney, according to the following formula:

\[
\text{CRG} = 100 - \left(\frac{\text{left KW} - \text{right KW}}{\text{right KW}}\right) \times 100
\]

Kidney protein and DNA were assayed in tissue homogenates (24,25), and the total kidney protein and total kidney DNA were calculated.

Statistical Analysis

Results were expressed as mean ± SEM and compared using t test on Excel software. \( P < 0.05 \) was considered significant.

Results

Opossum Kidney Cell Culture

Incubation of OK cells with rhIGF-I 50 ng/ml produced a 38% increase in the incorporation of [³H] thymidine into DNA. The biologic efficacy of JB3 as an IGF-I antagonist is shown in Figure 2. Preincubation with JB3 for 3 h inhibited the increase in radiolabeled thymidine incorporation into DNA induced by rhIGF-I 50 ng/ml (IC₅₀ = 0.15 μg/ml). JB3 showed no evidence of agonist activity at higher concentrations; 100% inhibition was demonstrated over the range 5 to 50 μg/ml.

STZ-Induced Diabetes

Measurements of blood glucose, body weight, and kidney growth obtained 4 d after the administration of STZ or citrate buffer are shown in Table 1. STZ-induced diabetic rats had a three- to fourfold increase in blood glucose concentration together with fall in body weight. Kidney wet weight, protein, and DNA all were significantly higher in STZ-induced diabetic rats than in nondiabetic controls (\( P < 0.01 \)) by a mean of 24%, whereas the DNA/protein ratio remained unchanged.

The effect of either saline vehicle or a maximum inhibitory dose of JB3 (3 μg/kg per day) on body weight, kidney weight, total kidney protein, and total kidney DNA in citrate buffer and STZ-injected rats is also shown in Table 1. In STZ-induced diabetic rats, JB3 (3 μg/kg per day) had no effect on either the elevated blood glucose concentration or the decrease in rat body weight, but a significant decrease in the elevated kidney wet weight, total kidney protein, and total kidney DNA was observed (Figure 3). The DNA/protein ratio remained unchanged. In the diabetic rat, JB3 3 μg/kg per day did not inhibit food consumption (30.6 ± 1.2 versus 28.9 ± 1.4 g/24 h), whereas in nondiabetic rats, no significant change in any measured parameter was detected in animals that received JB3 (3 μg/kg per day).

The dose-response relationship for the inhibitory effect of JB3 on kidney wet weight, protein, and DNA is shown in Figure 4. The increase in kidney wet weight, DNA, and protein content in the STZ-induced diabetic rat was reduced by JB3 (1 to 10 μg/kg per day) and was abolished at the maximum inhibitory dose of 3 μg/kg per day. Studies with higher doses of JB3 showed the dose-response curve of the inhibitory effect of JB3 on kidney growth to be bell-shaped with no significant inhibition of the increase in kidney wet weight, DNA, or
Table 1. Effect of JB3 (3 μg/kg per day) on body weight, blood glucose, and kidney growth of male Wistar rats 4 d after either intravenous STZ 60 mg/kg or CB.

<table>
<thead>
<tr>
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<th>JB3</th>
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<td>STZ</td>
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<td></td>
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<td></td>
</tr>
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<td>STZ (day 0)</td>
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<td>366 ± 6</td>
<td></td>
<td>359 ± 20</td>
<td>369 ± 11</td>
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<td>terminal (day 4)</td>
<td>385 ± 10</td>
<td>346 ± 6b</td>
<td>&lt;0.0001</td>
<td>371 ± 21</td>
<td>349 ± 12b</td>
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<td>Blood glucose (mmol/l)</td>
<td>5.4 ± 0.2</td>
<td>17.6 ± 2.1</td>
<td>&lt;0.0001</td>
<td>5.6 ± 0.3</td>
<td>19.8 ± 2.8</td>
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<td>Kidney wet weight (g)</td>
<td>1.31 ± 0.05</td>
<td>1.59 ± 0.07</td>
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<td>1.24 ± 0.07</td>
<td>1.36 ± 0.08</td>
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<tr>
<td>Tissue water (%)</td>
<td>79.7 ± 0.4</td>
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<td>NS</td>
<td>81.1 ± 0.9</td>
<td>78.9 ± 0.7</td>
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<td>Fractional kidney wet weight (mg/g body weight)</td>
<td>3.48 ± 0.08</td>
<td>4.58 ± 0.15</td>
<td>&lt;0.0001</td>
<td>3.33 ± 0.06</td>
<td>3.96 ± 0.22</td>
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<td>Total kidney protein (mg)</td>
<td>1.36 ± 6</td>
<td>172 ± 13</td>
<td>&lt;0.02</td>
<td>137 ± 11</td>
<td>139 ± 6</td>
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<tr>
<td>Total kidney DNA (mg)</td>
<td>1.51 ± 0.12</td>
<td>2.07 ± 0.04</td>
<td>&lt;0.05</td>
<td>1.44 ± 0.10</td>
<td>1.59 ± 0.08</td>
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<tr>
<td>DNA/protein (μg/mg)</td>
<td>11.9 ± 1.1</td>
<td>12.2 ± 0.7</td>
<td>NS</td>
<td>10.6 ± 0.4</td>
<td>11.5 ± 0.7</td>
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</tbody>
</table>

* Results are expressed as mean ± SEM; n = 6 per group; STZ, streptozotocin; CB, citrate buffer.

b P < 0.05 compared with day 0.

Figure 3. Effect of JB3 3 μg/kg per day (■) compared with saline vehicle (□) on total kidney protein and DNA 4 d after the intravenous administration of either streptozotocin (STZ) 60 mg/kg or citrate buffer (CB) to 12-wk-old male Wistar rats. Vertical bars indicate SEM; n = 6 per group.

protein being observed at a dose of 30 μg/kg per day. Importantly, JB3 had no effect on hyperglycemia in the STZ-induced diabetic rat at any dose studied (1 to 30 μg/kg per day).

Unilateral Nephrectomy

CRG measured in UNx animals 11 d after nephrectomy was 56.7 ± 2.4%. Kidney wet weight, protein, and DNA all were significantly higher (Table 2) in kidneys obtained from UNx than sham-operated animals, together with a significant increase in the DNA/protein ratio (P < 0.01).

The effect of a maximum inhibitory dose of JB3 (10 μg/kg per day) on body weight, kidney wet weight, total kidney

Figure 4. Dose-response relationship for the inhibitory effect of JB3 on the increase in kidney wet weight (▲), protein (■), and DNA (●) 4 d after the induction of diabetes using STZ in the Wistar rat. Dose-response curves were constructed using mean values obtained from the left kidney calculated as [diabetic (JB3) − nondiabetic (saline)/diabetic (saline) − nondiabetic (saline)] × 100. All data points represent the mean of experiments performed in six animals.
protein, and DNA measured in the left kidney of UNx and sham-operated rats 11 d after surgery is shown in Table 2. In UNx rats, JB3 (10 μg/kg per day) produced a significant decrease in kidney wet weight, protein, and DNA in the absence of any decrease in food consumption (24.0 ± 0.2 versus 23.9 ± 0.2 g/24 h), although the elevated renal DNA/protein ratio remained unchanged. CRG was lower than in animals that received the saline vehicle; the lowest value of 36 ± 6% was achieved at a dose of JB3 (10 μg/kg per day), representing a decrease in CRG of 41% (P < 0.01). Unlike the STZ-induced diabetic rat, the maximum inhibitory dose of JB3 (10 μg/kg per day) did not abolish the increase in kidney growth; a significant increase in kidney wet weight and DNA but not in kidney protein was still observed. In addition, in sham-operated rats that were treated with JB3 (10 μg/kg per day), the mean kidney wet weight, total kidney DNA, and protein all were lower than those in rats that received the saline vehicle (Figure 5), although the only difference that reached statistical significance was in total kidney protein (159 ± 4.6 versus 180 ± 9.8 mg/kidney; P < 0.05).

The dose-response relationship for the inhibitory effect of JB3 on the increase in kidney wet weight, total kidney protein, and total kidney DNA after UNx is shown in Figure 6. The increase in kidney wet weight, protein, and DNA content in the UNx rats was reduced by JB3 (5 to 10 μg/kg per day) maximum inhibition for each parameter being seen at a dose of 10 μg/kg per day. No change in tissue water content was observed at any dose studied, which remained constant at 77%. Studies with higher doses of JB3 showed the dose-response curve of the inhibitory effects of JB3 to be bell-shaped. At the higher dose of 30 μg/kg per day, the inhibition of CRG by JB3, although still significant (P < 0.05), had declined to 27%.

The effect of JB3 (10 μg/kg per day) on normal kidney growth was assessed by comparing the wet weight of right kidneys removed from UNx rats at nephrectomy (day 0) with those removed from sham-operated animals at sacrifice (day 11). The results are shown in Figure 7. In rats that were treated with saline vehicle, the wet weight of the right kidney was significantly higher at day 11 compared with day 0 (P < 0.005). In rats that were treated with JB3 10 μg/kg per day, no significant increase in the wet weight of the right kidney was observed during the same 11-d period.

Table 2. Effect of JB3 (10 μg/kg per day) on kidney growth in the rat 11 d after either UNx or sham operation

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<tr>
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<tr>
<td></td>
<td>Sham</td>
<td>UNx</td>
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<td>Body weight (g)</td>
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<tr>
<td>pump (day −3)</td>
<td>180 ± 2</td>
<td>177 ± 2</td>
<td>NS</td>
<td>180 ± 2</td>
<td>175 ± 2</td>
<td>NS</td>
</tr>
<tr>
<td>surgery (day 0)</td>
<td>206 ± 3</td>
<td>228 ± 4</td>
<td>NS</td>
<td>216 ± 2</td>
<td>222 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>terminal (day 11)</td>
<td>271 ± 5</td>
<td>252 ± 5</td>
<td>NS</td>
<td>271 ± 4</td>
<td>259 ± 6</td>
<td>NS</td>
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<tr>
<td>Kidney wet weight (g)</td>
<td>1.10 ± 0.04</td>
<td>1.53 ± 0.07</td>
<td>&lt;0.001</td>
<td>1.05 ± 0.04</td>
<td>1.30 ± 0.05</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Tissue water (%)</td>
<td>77.4 ± 0.6</td>
<td>77.5 ± 0.8</td>
<td>NS</td>
<td>77.4 ± 0.3</td>
<td>77.1 ± 0.9</td>
<td>NS</td>
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<tr>
<td>Fractional kidney wet weight (mg/g body weight)</td>
<td>4.05 ± 0.14</td>
<td>6.08 ± 0.33</td>
<td>&lt;0.001</td>
<td>3.87 ± 0.15</td>
<td>5.22 ± 0.29</td>
<td>&lt;0.005</td>
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<td>Compensatory kidney growth (%)</td>
<td>—</td>
<td>56.7 ± 2.4</td>
<td></td>
<td>—</td>
<td>30.0 ± 6.4b</td>
<td></td>
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<td>Total kidney protein (mg)</td>
<td>180 ± 9.8</td>
<td>235 ± 9.8</td>
<td>&lt;0.005</td>
<td>159 ± 4.6</td>
<td>180 ± 10.5</td>
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<td>Total kidney DNA (mg)</td>
<td>2.30 ± 0.09</td>
<td>4.13 ± 0.24</td>
<td>&lt;0.001</td>
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<td>DNA/protein (μg/mg)</td>
<td>12.9 ± 0.5</td>
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<td>&lt;0.01</td>
<td>13.8 ± 0.9</td>
<td>17.8 ± 1.7</td>
<td>&lt;0.05</td>
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</table>

* Results are expressed as mean ± SEM; n = 6 per group; UNx, unilateral nephrectomy.

b P < 0.01 compared with saline vehicle

Figure 5. Effect of JB3 10 μg/kg per day (□) compared with saline vehicle (□) on total kidney protein and DNA 11 d after either unilateral nephrectomy (UNx) or sham operation (Sham) in 8-wk-old male Wistar rats. Vertical bars indicate SEM; n = 6 per group.

Discussion

A selective IGF-I receptor antagonist was used to determine the contribution of IGF-I to kidney growth in the rat after UNx and the induction of diabetes. JB3, the IGF-I receptor antagonist used, was a cyclic 12 amino acid peptide generated by
solid phase peptide synthesis using d-isomer amino acids and purified by high-pressure liquid chromatography (21). The presence of the amino acid cysteine at positions 1 and 12 allowed oxidation to form a cyclic peptide through cysteine-cysteine bond formation, confirmed by mass spectrometry. IGF-I receptor antagonism was determined in vitro using an opossum kidney tubular cell line where preincubation with JB3 (0.5 to 50 μg/ml) prevented the increased incorporation of [3H] thymidine into DNA induced by rhIGF-I. In previous studies, using 3T3 fibroblasts transfected with the human IGF-I receptor protein, JB3 was shown to be selective for the IGF-I system, inhibiting autophosphorylation of the IGF-I receptor but having no effect on autophosphorylation of the epidermal growth factor receptor or activation of phospholipase Cγ1 by platelet-derived growth factor (PDGF) (21). In the present study, renal growth was inhibited to a different extent by the subcutaneous infusion of JB3 after either the induction of diabetes or UNx. The results are summarized in Figure 8.

In the STZ rat, complete inhibition of diabetic kidney growth was observed at a JB3 dose of 3 μg/kg per day. JB3 administration had no effect on the hyperglycemia of experimental diabetes and did not inhibit food consumption by the diabetic rats. In this study, kidney growth (24%) was measured 4 d after the induction of diabetes; no change in the DNA/protein ratio was detected. Four d is required for the maximum increase (fourfold) in the kidney content of IGF-I to develop after the induction of diabetes in our adult Wistar rat colony (6). Changes in the IGF-I pathway previously shown to follow the induction of diabetes in the STZ-induced diabetic rat include (1) a decrease in the serum IGF-I concentration (8), (2) a transient increase in renal IGF-I protein (4), (3) an increase in IGF-I receptor mRNA and protein (10,11), and (4) a shift in the balance of renal IGFBP-1 from medulla to cortex and in IGFBP-5 from cortex to medulla (12). However, the changes in renal IGF-I biochemistry that follow the induction of diabetes must be interpreted with caution because an increase in tissue sensitivity to IGF-I is also an important facet of the growth response (26,27). As a result of the transient nature of the increase in renal IGF-I protein, any role for IGF-I in the maintenance as well as in the initiation of renal growth has been called into question (28), although signal transduction by the IGF-I pathway could be maintained through an increase in IGF-I receptor protein (10). In type 1 diabetic patients (mean duration, 7 yr), kidney volume showed a strong positive core-
relation with urinary IGF-I excretion (29), whereas in other models of experimental diabetes, such as the nonobese diabetic mouse, renal hypertrophy is associated with a persistent renal accumulation of IGF-I (30). IGF-I and IGF-I receptor mRNA are also upregulated in diabetic glomerular mesangial cells (31,32) where a separate role for IGF-I as a mediator of diabetic hyperfiltration has been proposed, independent of kidney growth (33).

In the UNx rat, the maximum effect of JB3 on kidney growth was observed at a dose of 10 μg/kg per day whereby CRG was inhibited by 60%, without any effect on food consumption. In this study, kidney growth (55%) was measured 11 d after UNx in 8-wk-old Wistar rats. The DNA/protein ratio, which increased after nephrectomy, remained unaffected by JB3 administration. Changes in renal IGF-I biochemistry reported to follow a reduction in renal mass include (1) no change in the serum IGF-I concentration, (2) a transient increase in renal IGF-I protein particularly in the collecting duct (4,28), (3) no change in either serum or kidney IGF binding proteins 1 to 4 (14), whereas (4) an increase, a decrease, and no change in renal IGF-I mRNA all have been reported (16,34,35). Unlike diabetes, transcriptional changes in IGF-I and its receptor protein have been described in prepubertal rather than postpubertal rats in the 48-h period after UNx (15,17). In a more recent study in weanling rats, an increase in mRNA for IGFBP 3, 4, and 5 was observed 7 d after UNx, whereas IGF-I mRNA, protein, and receptor binding remained unchanged (16). Studies performed in the isolated rat kidney have also proposed a role for IGF-I as a mediator of compensatory hyperfiltration after UNx, independent of kidney growth (36).

Because both kidney growth and the renal content of IGF-I can be inhibited by somatostatin (37), a role for GH in activating renal IGF-I has frequently been proposed. An IGF-I antagonist, such as JB3, would be expected to inhibit any properties of GH mediated through the IGF-I pathway but cannot distinguish between systemic or renal activation. Studies using a GH-releasing factor antagonist suggest a role for GH in early kidney growth after UNx in the mature rat (17), and in the adult mouse, kidney growth after UNx was inhibited by the GH receptor antagonist G120K-PEG (38). In previous studies from our own laboratory, diabetic kidney growth was markedly blunted in the Lewis dwarf rat (deficient in GH), suggesting a GH-dependent response (39). In contrast, CRG to a reduction in renal mass in the Lewis dwarf rat was similar to that detected in the Lewis base strain with normal GH secretion, indicating a GH-independent response (19). The UNx study yielded tentative evidence, however, that the kidney growth that occurs with age (known to be GH dependent) could also be inhibited by JB3. Any involvement of GH in the 40% of CRG after UNx unaffected by JB3 would require a mechanism independent of IGF-I.

In both diabetic and UNx rat models, the dose-response curves of the inhibitory effect of JB3 on kidney growth were bell-shaped. A similar bell-shaped dose-response curve of the inhibitory effect of JB3 on vascular smooth muscle proliferation has previously been described in the rat after balloon damage to the carotid artery (22). This raises the possibility that JB3 may be a partial agonist at the IGF-I receptor possessing mixed agonist/antagonist activity. However, in opossum kidney cells in culture, the concentration/response curve of the inhibitory effect of JB3 on thymidine incorporation into DNA showed a sigmoidal relationship, 100% inhibition occurring over the higher concentration range of 0.5 to 50 μg/ml, with no evidence of agonist activity. In the absence of species differences, the presence of a bell-shaped growth inhibition curve for JB3 in the diabetic and UNx rat should be explained by an “in vivo phenomenon,” possibly mediated through changes in GH or the IGF type 2 receptor. GH could be involved because the pituitary release of GH is inhibited by plasma IGF-I (40). JB3 is not selective for the IGF-I receptor in the kidney and would be expected to produce systemic receptor inhibition. IGF-I receptor antagonism induced by JB3 might therefore increase plasma GH-enhancing mitogenic signaling to the kidney. A second possibility is associated with the selectivity of JB3 for the IGF type 1 rather than the IGF type 2 receptor. In the presence of IGF type 1 receptor blockade,
kidney growth could be enhanced through the IGF type 2 receptor upregulated after either UNx (41) or diabetes (42).

Evidence of a predominant role for IGF-I in initiating the kidney growth that follows either UNx or the induction of diabetes does not preclude the involvement of other renal growth factors, in addition to GH. CRG, for example, may be the consequence of an increase in the gene expression for both positive and negative growth control elements (43), and roles for epidermal growth factor (44), hepatocyte growth factor (45), platelet-derived growth factor (46), transforming growth factor β (47), angiotensin II (48), and atrial natriuretic peptide (49) have also been proposed. However, the mitogenic signaling of other growth factors may also require the presence of the IGF-I receptor (26). Epidermal growth factor, for example, can stimulate IGF-I gene expression (50), whereas PDGF can increase the activity of a promoter site for the IGF-I receptor gene (51). Growth factors that are expressed in the kidney but do not require the presence of the IGF-I receptor include the granulins (52), which could mediate any kidney growth unaffected by JB3 administration.

In conclusion, studies with the IGF-I receptor antagonist JB3 support the concept of IGF-I as an important mediator of the early kidney growth after the induction of diabetes or a reduction in renal mass. Kidney growth may be an important stimulus in the development of chronic renal failure (53). Because IGF-I can directly stimulate renal matrix proteins (54), studies on the effect of JB3 in the development of kidney scarring are in progress.

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References

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