# Octreotide Prevents the Early Increase in Renal Insulin-Like Growth Factor Binding Protein 1 in Streptozotocin Diabetic Rats

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**The early renal growth in streptozotocin (STZ)-induced diabetic rats is preceded by a transient rise in renal tissue insulin-like growth factor (IGF)-I concentration. Administration of the long-acting somatostatin analog octreotide to STZ diabetic rats inhibits the early increase in kidney IGF-I and the increase in kidney size without affecting metabolic control. We studied the effects of octreotide treatment on the intrarenal IGF axis at 2 and at 7 days after the induction of STZ diabetes. Two days after induction of diabetes, kidney IGF-I was increased from 850 ± 43 ng/g tissue in controls to 1,648 ± 165 ng/g tissue ($P < 0.001$) in diabetic animals. The diabetes-associated increase in renal IGF-I 48 h after STZ injection was totally prevented by octreotide (IGF = 780 ± 57 ng/g tissue). However, 7 days after the induction of diabetes, kidney IGF-I was similar to that of control and was not affected by octreotide. No difference in serum IGF-I was observed between controls and diabetic rats after 2 days of diabetes; however, octreotide treatment resulted in a significant decrease of serum IGF-I after 2 days when compared with control rats ($P < 0.05$). Renal IGF-I mRNA was significantly decreased to the same extent in both diabetic groups 2 and 7 days after the induction of diabetes, while renal IGF-I receptor (IGF-IR) mRNA was unchanged in rats from either group. Two days after induction of diabetes, renal insulin-like growth factor binding protein (IGFBP)-1 mRNA and 30-kDa IGFBPs (containing IGFBP-1) increased by 186 and 192%, respectively, in untreated diabetic animals compared with controls. Octreotide treatment prevented the diabetes-associated rise in renal IGFBP-1 mRNA and protein. However, 7 days after the induction of diabetes, renal IGFBP-1 mRNA and protein were similarly increased in both octreotide-treated or untreated diabetic rats. Renal IGFBP-3 gene expression and protein and IGFPB-5 mRNA remained unchanged after 2 and 7 days of diabetes when treated or untreated with octreotide. We conclude that the well-known inhibitory effect of octreotide on the early increase in renal IGF-I concentration and renal size in diabetes may be mediated through a direct effect on renal IGFBP-1 levels. *Diabetes* 47:924–930, 1998**

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GH, growth hormone; IGF, insulin-like growth factor; IGFBP, IGF binding protein; IGF-IR, IGF-I receptor; NIH, National Institutes of Health; RIA, radioimmunoassay; STZ, streptozotocin.

The early and rapid increase in renal growth and function seen in streptozotocin (STZ)-induced diabetic rats is preceded by a rise in renal tissue concentration of insulin-like growth factor (IGF)-I, reaching a peak at 24–48 h after the induction of diabetes and returning to baseline levels after 4 days (1). Diabetic dwarf rats, with growth hormone (GH) and IGF-I deficiency, exhibit a diminished renal hypertrophy as well as a smaller rise in kidney IGF-I than in diabetic controls with an intact pituitary gland (2).

The rise in endogenous kidney IGF-I during early STZ-induced diabetes occurs in the presence of either decreased or unchanged renal IGF-I mRNA and decreased or unchanged serum IGF-I (3). The early increase in renal IGF-I in STZ diabetes cannot be related to the IGF-I receptor since in early experimental diabetes, no changes are observed in renal IGF-I receptor mRNA or receptor binding (4,5). Concomitantly, with the increase in endogenous kidney IGF-I in early STZ diabetes, change in IGF binding protein (IGFBP) gene expression and proteins are seen mainly as an increase in cortical IGFBP-1 and to a lesser extent, medullary IGFBP-5 (6–8). Renal IGFBP-3 gene expression remained unchanged (6) or increased (8). This finding corroborates with the finding of increased IGF-I binding in the diabetic kidney cortex to low molecular weight material that represents IGFBP-1 (8). The rapid increase in kidney IGFBP-1 after the induction of STZ diabetes seems to play a major role in the early increase of IGF-I in the STZ-induced diabetic kidney, which is followed by kidney hypertrophy. The IGFBPs act as carriers for IGFs; however, they may act as local modulators of IGF action. In particular, such mechanisms may be operative for IGFBP-1 since it contains an Arg-Gly-Asp motif near the COOH-terminal, which enables it to interact with cell surfaces and deliver IGFs to adjacent IGF receptors, thus enhancing the subsequent binding and action of IGFs on the cell (9).

Administration of the long-acting somatostatin analog octreotide to STZ diabetic rats has been shown to inhibit the early increase in kidney IGF-I and to prevent the increase in kidney size without affecting metabolic control (10). However, the mechanism by which octreotide prevents the increase in kidney IGF-I and kidney size during the early phase of diabetes is still unknown.

Accordingly, the aim of the present study was to examine the effects of octreotide treatment on the IGFBPs gene expression and proteins concentrating on renal IGFBP-1 mRNA and protein levels at 2 and 7 days after induction of STZ diabetes, at the time when the most pronounced changes in renal IGF-I and size occur.

## RESEARCH DESIGN AND METHODS

**Animals.** Hebrew University Sabra male rats with a body weight range of 180–200 g were studied. The animals were maintained and treated in accordance with the regulations of the Hadassah Committee on Animal Care and Use. Rats were housed in metabolic cages beginning 3 days before the induction of diabetes. During the experiments, the rats had free access to standard rat diet and tap water. After an overnight fast, rats were administered a single intraperitoneal (i.p.) injection of STZ (65 mg/kg body wt i.p. in 0.01 mol/l citrate buffer, pH 4.0) or vehicle. Administration of STZ resulted in the induction of hyperglycemia in 90% of the animals 24 h after treatment. Metabolic status of the animals was monitored 1, 2, and 7 days after STZ injection by estimating levels of blood glucose, urinary glucose, and ketones (Glucostix, Diastix, and Ketostix, respectively) (Ames, Elkhart, IN). Rats with blood glucose >15 mmol/l 24 h after STZ injection and >18 mmol/l after 48 h, with urine glucose levels >100 mmol/l 48 h after STZ injection, and an absence of ketonuria were used in the study.

**Study design**

***Octreotide injection to diabetic rats.*** Octreotide (Sandoz, Basle, Switzerland) at 100 µg twice daily was injected subcutaneously to half of the STZ-injected diabetic rats ($n$ = 30) beginning immediately after the injection of STZ. The other half ($n$ = 30) were injected with a similar volume of saline. Approximately half of the rats were killed 48 h after the induction of diabetes ($n$ = 12 and 13 rats from the octreotide-treated and untreated diabetic rats, respectively), while the remainder of the rats that developed diabetes were killed 7 days after the induction of diabetes ($n$ = 13 and 12 for octreotide-treated or untreated diabetic rats, respectively). Control age- and sex-matched nondiabetic rats were injected with a similar volume of saline and killed at 48 h ($n$ = 12) and at 7 days ($n$ = 12) after the beginning of the study. When killed, blood was drawn from the aorta. The kidney and liver were resected, weighed, and immediately frozen in liquid nitrogen and then kept in –70°C until analyzed. In six rats from each group, one kidney was separated into cortex and medulla and then frozen to –70°C.

***Octreotide injection to normal rats.*** Octreotide at 100 µg (Sandoz) twice daily was injected subcutaneously to half the rats ($n$ = 12), while the other half ($n$ = 12) was injected with saline. Rats were killed 24 h ($n$ = 6 in each group) and 48 h ($n$ = 6 in each group) after the first injection of octreotide. Blood and tissue were taken as in the diabetic rats.

**Kidney IGF-I extraction and IGF-I radioimmunoassay (RIA).** IGF-I extraction was performed according to D'Ercole (11) as previously described (3). Briefly, the kidneys were homogenized on ice in 1 mol/l acetic acid (5 ml/g tissue) with an Ultra Turrax TD 25 and further disrupted using a Potter Elvehjelm homogenizer. The tissues were extracted twice. After lyophilization, the samples were redissolved in 40 mmol/l phosphate buffer (pH 8.0). Tissue extracts were kept at –20°C until IGF-I assay was performed in diluted extracts. A linear relationship was found between biosynthetic IGF-I and IGF-I immunoreactivity of kidney and liver extracts at multiple concentrations, indicating antigen similarity and also that no binding proteins or receptors from the extracts interfered in the RIA. The measured kidney IGF-I concentrations were all corrected for serum IGF-I entrapped in the kidney tissue as previously described (12).

Serum IGF-I was measured after extraction with acid-methanol (30 µl serum and 750 µl acid methanol) (13). The mixture was incubated for 2 h at room temperature, centrifuged, and 25 µl of the supernatant was diluted 1:200 before analysis. Serum IGF-I was measured by RIA using a polyclonal rabbit antibody (Nichols Institute Diagnostics, San Capistrano, CA) and recombinant human IGF-I as standard (Amersham International, Amersham, Bucks, U.K.).

Mono-iodinated IGF-I ($^{125}$I-[Tyr$^{31}$]-IGF-I) was obtained from Novo Nordisk A/S Bagsvaerd, Denmark. When exposing the serum extract to Western ligand blotting, no IGFBPs could be identified; furthermore, semilog linearity of biosynthetic IGF-I and serum extracts was seen, indicating antigen similarity and also that no IGFBPs interfered in the RIA. Intra- and interassay coefficient of variation were below 5 and 10%, respectively.

**Gene expression of IGF-I, IGF-IR, IGFBP-1, and IGFBP-3 measured by solution hybridization–RNase protection assay.** Total RNA was prepared from renal tissue of individual rats by Tri Reagent (Molecular Research Center, Cincinnati, OH) according to the method of Chomczynski (14) and quantified by absorbance at 260 nm. The integrity of the RNA and the accuracy of the spectrophotometric determinations were assessed by visual inspection of the ethidium bromide–stained 28S and 18S ribosomal RNA bands after agarose formaldehyde gel electrophoresis of 10 µg aliquots, as described previously (15). The IGF-I, IGF-I receptor (IGF-IR), IGFBP-1, and IGFBP-3 riboprobes were generous gifts from Dr. LeRoith (National Institutes of Health [NIH], Bethesda, MD).

The antisense RNA probe used to detect IGF-IR mRNA has been previously described (16). This transcript contains 40 bases of vector sequence and 265 bases complementary to 15 bases of 5′-untranslated sequence and to the region that encoded the signal peptide and the first 53 amino acids of the IGF-IR $\alpha$ subunit. On hybridization of this RNA probe with IGF-IR RNA and subsequent RNase digestion, a protected band of 265 bases was obtained.

The riboprobe used to measure the levels of IGF-I mRNA has been described (17). The probe allows for the detection of both IGF-I mRNA species encoding IGF-Ia and IGF-Ib prohormones. Only the levels of IGF-Ia mRNA, which constitute >90% of the total IGF-I message and correlate with the levels of IGF-Ib mRNA, were measured in this study.

The IGFBP-1 mRNA was measured using an antisense probe derived from a rat IGFBP-1 cDNA clone isolated from a dexamethasone-treated H-4 11-E-C3 hepatoma cell library (NIH) (G.T. Ooi, unpublished observations). The size of the protected band obtained by hybridizing this antisense RNA probe with IGFBP-1 mRNA was 203 bases.

The IGFBP-3 mRNA measurement has been previously described (18). The size of the protected band obtained by hybridizing this antisense RNA probe with IGFBP-3 mRNA was 493 bases.

The pT7 RNA 18S (Ambion, Austin, TX) was used as antisense control. The size of the protected fragment was 80 bases. Solution hybridization–RNase protection assays were performed as described (16). Briefly, 20 µg of total RNA were hybridized with $1 \times 10^6$ dpm $^{32}$P-labeled antisense RNA probes, and 1 ng of total RNA was hybridized with 18S antisense RNA probe. The hybridization was carried out at 45°C for 16 h in a buffer containing 80% formamide. After hybridization, RNA samples were digested with RNase A and T1 and the protected hybrids were extracted with phenol-chloroform and ethanol precipitated, and electrophoresed on 8% polyacrylamide-8M urea denaturing gel. Multiple autoradiograms from each gel were scanned by a densitometer connected to an Apple Macintosh computer. Changes in the signals were expressed as the ratio between experimental and control values.

**Gene expression of IGFBP-5 measured by Northern blot hybridization.** Of the total RNA, 20 µg were electrophoresed on 1.3% agarose/2.2 mol/l formaldehyde gels in 3-morpholinopropanesulphonic acid buffer. The RNA was then transferred onto MagnaGraph (MSI, Westboro, MA) nylon membranes and was cross-linked to the membranes with a UV cross-linker (Hoefer Scientific Instrument, San Francisco, CA).

A 6-kb transcript encoding the IGFBP-5 cDNA was detected by using a 300-bp *Sac*II-*Hind*III fragment from the rat IGFBP-5 subcloned into pRBP5SH. This construct was linearized with *Sac*II and radiolabeled with [$\alpha$-$^{32}$P]dCTP 3,000 Ci/mmol (Amersham) by random primed DNA labeling kit (Boehringer Mannheim, Mannheim, Germany).

TABLE 1
Serum levels of glucose, insulin, GH, IGF-I, and octreotide in nondiabetic control, untreated diabetic, and octreotide-treated diabetic animals

| | Day 2 | | | Day 7 | | |
|---|---|---|---|---|---|---|
| | Control | Diabetes | Diabetes + octreotide | Control | Diabetes | Diabetes + octreotide |
| $n$ | 12 | 12 | 13 | 12 | 13 | 12 |
| Glucose (mmol/l) | 7.3 ± 0.3 | 29.9 ± 0.9* | 26.8 ± 0.5† | 6.8 ± 0.3 | 26.8 ± 1.1* | 27.1 ± 0.4† |
| Insulin (mU/l) | 55.0 ± 4.6 | 18.0 ± 5.0* | 22.0 ± 9.8† | 61.9 ± 12.4 | 11.0 ± 2.1* | 9.0 ± 3.3† |
| GH (µg/l) | 42.0 ± 11.0 | 39.0 ± 12.0 | 26.0 ± 2.0†‡ | 62 ± 14 | 21 ± 6* | 24 ± 8† |
| IGF-I (µg/l) | 526 ± 43 | 454 ± 88 | 289.0 ± 26.0†‡ | 586 ± 33 | 271 ± 17* | 260 ± 22† |
| Octreotide (ng/l) | 0 | 0 | 101 ± 20 | 0 | 0 | 118 ± 17 |

Data are means ± SE. *Control versus diabetes; †control versus diabetes + octreotide; ‡diabetes versus diabetes + octreotide. $P < 0.05$.

TABLE 2
Serum 30-kDa IGFBPs, IGFBP-3, and IGFBP-4 in nondiabetic control, untreated diabetic, and octreotide-treated diabetic animals

| | Day 2 | | | Day 7 | | |
|---|---|---|---|---|---|---|
| | Control | Diabetes | Diabetes + octreotide | Control | Diabetes | Diabetes + octreotide |
| *n* | 12 | 12 | 13 | 12 | 13 | 12 |
| 30-kDa IGFBPs (AU/mm$^2$) | 4.0 ± 1.2 | 29.0 ± 16.0* | 51.0 ± 11.0† | 11.0 ± 1.9 | 12.5 ± 1.8 | 16.7 ± 4.3 |
| IGFBP-3 (AU/mm$^2$) | 94.0 ± 24.0 | 93.0 ± 31.0 | 62.0 ± 12.00†‡ | 119.3 ± 8.6 | 40.6 ± 5.9* | 35.8 ± 3.7† |
| IGFBP-4 (AU/mm$^2$) | 39.0 ± 8.0 | 21.0 ± 6.0 | 13.0 ± 4.0† | 38.8 ± 5.1 | 21.1 ± 2.7* | 23.0 ± 2.1† |

Data are means ± SE. *Control versus diabetes; †control versus diabetes + octreotide; ‡diabetes versus diabetes + octreotide. The numbers are the relative density of the bands measured by Western ligand blotting. $P < 0.005$.

RNA hybridization was performed in a hybridization oven (Micro-4 Hybaid limited) at 65°C for 20 h in a hybridization solution (0.2 mmol/l $Na_2HPO_4$, pH 7.2, 7% [vol/vol] SDS, 1% [wt/vol] BSA and 1 mmol/l EDTA). The washing was done in 0.4 × SSC, and 0.1% SDS at 65°C. Gels were exposed to Kodak X-Omat AR film (Eastern Kodak, Rochester, NY) at –70°C with two intensifying screens, and the band (6 kb) on the autoradiography that corresponds to the IGFBP-5 was analyzed. The autoradiograms were quantitated using a phosphoimager (Imagequant; Molecular Dynamic, Sunnyvale, CA).

**Tissue extraction for IGFBPs.** Approximately 50 mg of thawed kidney or liver tissue was placed in 1.5-ml polypropylene tubes and weighed. The tissue was homogenized for 2 min in 0.5 ml of 20 mmol/l Tris-HCl (pH 7.4 containing 2% Triton X-100) using a micropestel (Research Products International, Mount Prospect, IL). After adding 0.13 ml of Laemmli buffer, each tube was boiled for 5 min and incubated overnight at 4°C. Aliquots of extracts were stored at –80°C. Protein content of the extracts was measured using a protein assay (Pierce Rockford, Rockford, IL) with bovine serum as standard.

**Western ligand blotting for IGFBPs in serum and kidney.** SDS-PAGE and Western ligand blotting were performed according to the method of Hossenlopp et al. (7). In brief, thawed extracts were boiled for 1 min and centrifuged for 1 min at 13,000 rev/min. An aliquot of the supernatant equivalent to 200 µg of tissue protein or 2 µl of serum was subjected to SDS-PAGE (10% polyacrylamide) under nonreducing conditions. The electrophoresed proteins were transferred by electroelution onto nitrocellulose paper (Schleicher and Schuell, Munich, Germany) and the membranes were incubated overnight at 4°C with ~$0.5 \times 10^6$ cpm $^{125}$I-IGF-I (specific activity 2,000 Ci · mmol$^{-1}$ · l$^{-1}$) in 10 ml 10 mmol/l Tris-HCl buffer containing 1% bovine serum albumin and 0.1% Tween (pH 7.4). Membranes were washed with Tris-HCl buffer, and after drying overnight, the nitrocellulose sheets were autoradiographed with Kodak X ray film and exposed to Du Pont-New England Nuclear enhancing screens at –80°C for 3–7 days. Western ligand blots were quantified by densitometry using a Shimadzu CS-9001 PC dual wavelength flying spot scanner.

**Biochemistry.** Plasma glucose concentration was measured by the glucose oxidase method (Boehringer Mannheim). Plasma insulin levels were determined by RIA using human insulin as standard (Sorin Beiomedica, Saluggia, Italy). Serum rat GH was measured by RIA purchased from Amersham. Serum octreotide was measured by RIA as previously described (19). Intra- and interassay coefficient of variations were <5% and 10% for all assays.

**Statistics.** Analysis of variance for repeated measurements was used in evaluation of differences in combination with a Bonferroni test for multiple comparisons and unpaired Student's test. A $P$ value of <0.05 was regarded as significant. Values are given as mean ± SE.

## RESULTS

**Metabolic parameters, body, and kidney weights.** At 24 h after STZ injection, mean blood glucose levels of the injected rats were 21.8 ± 2.2 mmol/l, increasing to 27.8 ± 1.6 mmol/l after 48 h. Data on serum glucose, insulin, IGF-I, IGFBPs, GH, and octreotide in nondiabetic rats, diabetic rats treated with saline, or octreotide on day 2 and 7 are given in Table 1 and 2. Administration of octreotide resulted in high serum levels of octreotide 12 h after the last injection. Octreotide injection to diabetic rats significantly decreased serum GH and IGF-I levels (Table 1) and serum IGFBP-3 and -4 levels (Table 2) after 2 days of diabetes.

The severity of diabetes was similar in both the octreotide-treated and untreated rats, as similar plasma glucose, insulin levels, and body weights were seen in the two groups (Tables 1 and 3). Kidney weight was significantly increased in the diabetic rats 7 days after STZ injection. Octreotide treatment, however, partially prevented the increase in kidney weight (Table 3).

**IGF system in the kidney.** Octreotide treatment in the diabetic rats prevented the early increase in renal IGF-I. However, 7 days after the induction of diabetes, renal IGF-I was similar in all three groups (Fig. 1).

Induction of STZ diabetes resulted in a significant decrease in renal IGF-I mRNA 75 ± 7% ($P < 0.05$) at 2 days and 75 ± 18% ($P < 0.05$) at 7 days after the induction of diabetes. Treatment with octreotide decreases renal IGF-I mRNA slightly but not significantly after 2 days (65 ± 5%) and 7 days (56 ± 9%) (Fig. 2).

Renal IGF-IR mRNA levels were unchanged after 2 days of diabetes (111 ± 8%), with a significant increase after 7 days (124 ± 7%) ($P < 0.05$) of diabetes. In octreotide-treated diabetic rats IGF-IR mRNA was unchanged at 2 days of diabetes (107 ± 7%) and was increased (141 ± 11%) ($P < 0.05$) after 7 days of diabetes. Renal IGFBP-1 mRNA increased significantly in

TABLE 3
Body and kidney weights in nondiabetic control, untreated diabetic, and octreotide-treated diabetic animals

| | Day 2 | | | Day 7 | | |
|---|---|---|---|---|---|---|
| | Control | Diabetes | Diabetes + octreotide | Control | Diabetes | Diabetes + octreotide |
| *n* | 12 | 12 | 13 | 12 | 13 | 12 |
| Body weight (g) | 191 ± 3.6 | 162.8 ± 3.2* | 167.6 ± 2.7† | 203 ± 2.8 | 195 ± 2.6* | 193 ± 1.4† |
| Kilodalton weight (g) | 1.711 ± 0.054 | 1.853 ± 0.047 | 1.755 ± 0.039 | 1.727 ± 0.023 | 2.273 ± 0.029* | 1.976 ± 0.031†‡ |

Data are means ± SE. *Control versus diabetes; †control versus diabetes + octreotide; ‡diabetes versus diabetes + octreotide. $P < 0.05$.

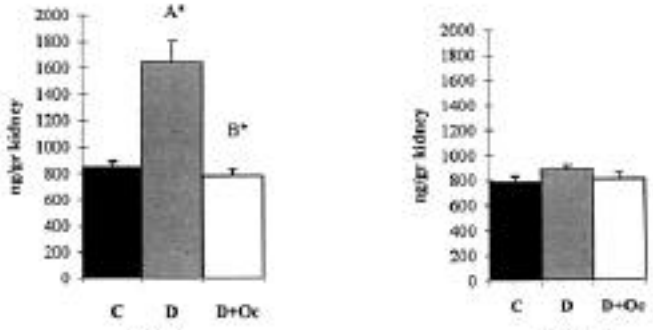


**FIG. 1. Kidney IGF-I in nondiabetic controls (C), untreated diabetic (D), and octreotide-treated diabetic animals (D + Oc) on day 2 and day 7 after induction of diabetes. Results are means + SE ($n$ = 6 for each group). A, control versus diabetes; B, diabetes versus diabetes + octreotide. *$P$ < 0.001.**

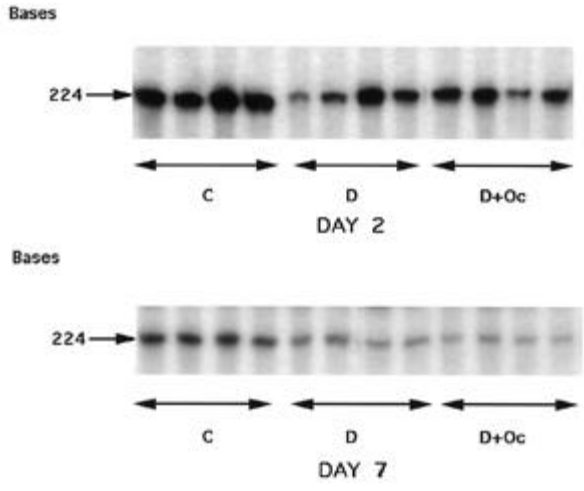


**FIG. 2. Expression of IGF-I mRNA in kidneys of nondiabetic controls (C), untreated diabetic (D), and octreotide-treated diabetic animals (D + Oc) on day 2 and day 7 after induction of diabetes. Renal levels of IGF-I mRNA were measured by solution hybridization RNase protection assays as described in METHODS. Autoradiograph obtained from four representative animals. The arrow on the left denotes the position and size of protected IGF-I probe band corresponding to IGF-I mRNA.**

the whole kidney and in the cortex and medulla of the diabetic rats. Treatment with octreotide prevented the increase in IGFBP-1 mRNA in the whole kidney (Figs. 3 and 4) after 2 days of diabetes but failed to do so after 7 days of diabetes. Octreotide also abolished the increased renal IGFBP-1 mRNA after 2 days when measured separately in the cortex ($P$ < 0.05), but not in the medulla. Renal 18S mRNA was similar in nondiabetic control rats (1,764 ± 120 densitometric units), untreated diabetic rats (2,160 ± 77 densitometric units), and octreotide-treated diabetic rats (1,950 ± 150 densitometric units). In accordance with this, octreotide treatment prevented the early increase in renal 30-kDa IGFBPs (containing IGFBP-1) in the kidney of the diabetic rats only after 2 days of diabetes (Figs. 3 and 5, Table 4). Correlations between kidney weight and 30-kDa IGFBPs were found after 7 days of diabetes at the time when octreotide was shown to prevent the diabetic-related increase in the kidney weight (Fig. 6).

At 2 and 7 days of diabetes, a nonsignificant decrease of renal IGFBP-3 mRNA levels (2 days 67 ± 18% and 7 days 72 ± 7% ) resulted. Treatment of the diabetic rats with octreotide did not change IGFBP-3 gene expression (2 days 88 ± 10% and 7 days 91 ± 5%). Renal IGFBP-3 protein was unchanged by the diabetic state after 2 and 7 days of diabetes, whether treated or untreated with octreotide (Table 4). In the diabetic kidney, octreotide treatment resulted in a significant decrease in renal IGFBP-4 after 2 days of diabetes (Table 4).

Renal IGFBP-5 mRNA was unchanged at 2 and 7 days after the induction of diabetes in rats treated or untreated with octreotide (2 days: diabetic rats 81 ± 13%, octreotide-treated diabetic rats 91 ± 6% ; 7 days: diabetic rats 91 ± 2%, octreotide-treated diabetic rats 99 ± 8%).

Treatment with octreotide of normal nondiabetic rats 24 and 48 h did not change renal IGFBP-1 gene expression (data not shown).

**IGF system in liver.** Hepatic IGF-I mRNA in diabetic and octreotide-treated diabetic animals did not change (data not shown). Liver IGFBP-1 mRNA was increased five- to sixfold in diabetic rats of both groups with 2 days of diabetes, with no difference between the two diabetic groups (Figs. 7 and 8).

Treatment with octreotide of normal nondiabetic rats 24 and 48 h resulted in a twofold increase in IGFBP-1 mRNA in the liver (data not shown).

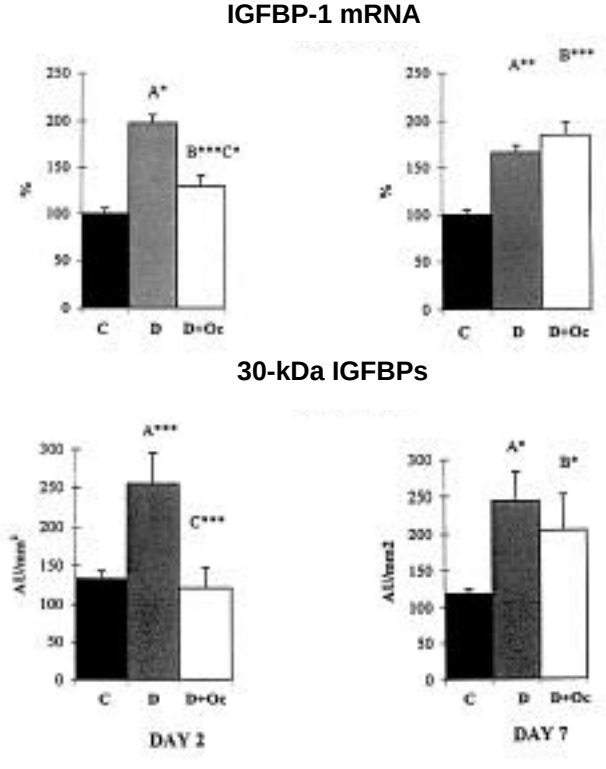


**FIG. 3. IGFBP-1 mRNA and 30-kDa IGFBPs 2 days after the induction of diabetes in the kidney of nondiabetic controls (C) ($n$ = 12 and $n$ = 6 rats, respectively), untreated diabetic (D) ($n$ = 12 and $n$ = 6 rats, respectively), and octreotide-treated diabetic rats (D + Oc) ($n$ = 13 and $n$ = 6 rats, respectively), and 7 days after induction of diabetes (C) ($n$ = 12 and $n$ = 6 rats, respectively) (D), ($n$ = 13 and $n$ = 6 rats, respectively), (D + Oc) ($n$ = 12 and $n$ = 6 rats, respectively). Results are means + SE. A, control versus diabetes; B, control versus diabetes + octreotide; C, diabetes versus diabetes + octreotide. *$P$ < 0.001; **$P$ < 0.01; ***$P$ < 0.05.**

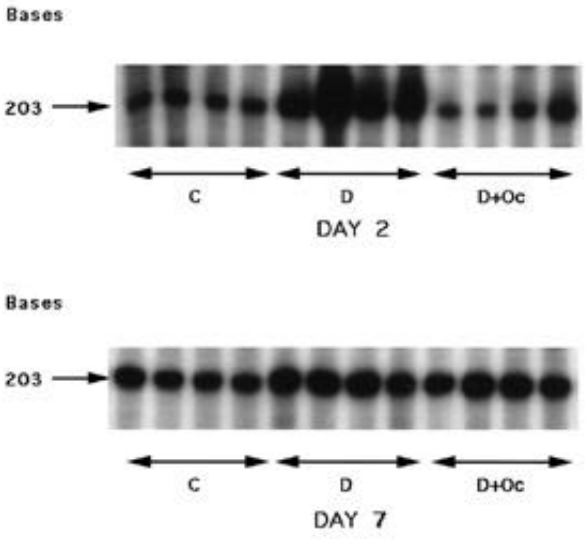


**FIG. 4. Expression of IGFBP-1 mRNA in kidneys of nondiabetic controls (C), untreated diabetic (D), and octreotide-treated diabetic animals (D + Oc) on day 2 and day 7 after induction of diabetes. Renal levels of IGFBP-1 mRNA were measured by solution hybridization RNase protection assays as described in METHODS. Autoradiograph obtained from four representative animals. The arrow on the left denotes the position and size of the probe band corresponding to IGFBP-1 mRNA.**

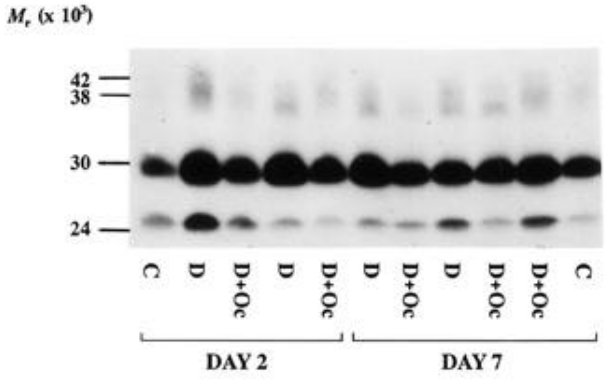


**FIG. 5. Representative Western ligand blot autoradiograph of kidney IGFBPs in nondiabetic controls (C), untreated diabetic (D), and octreotide-treated diabetic animals (D + Oc) on day 2 and day 7 after induction of diabetes. The IGFBPs appear from above: IGFBP-3 (38–42 kDa), 30-kDa IGFBPs, and IGFBP-4 (24 kDa).**

## DISCUSSION

The rapid increase in renal growth and function seen in STZ diabetes was preceded by an increase in renal tissue concentration of IGF-I, which is believed to act as a renotropic growth factor in early experimental diabetes. One possible explanation for early renal IGF-I accumulation in STZ diabetes may be increased local kidney IGF-I production. However, we demonstrated a tendency to decreased renal IGF-I mRNA levels in early STZ diabetes in line with previously published studies (3,6). Octreotide has previously been shown to prevent the GH–stimulated rise in serum IGF-I and hepatic IGF-I mRNA in nondiabetic hypophysectomized rats (20), suggesting a novel GH–independent downregulation of the IGF-I gene by octreotide. Thus the inhibitory effect of octreotide on the increase in renal IGF-I in the diabetic kidney could be the result of a further decrease in local IGF-I production in the kidney, secondary to further reduction in renal IGF-I mRNA. However, in our study the IGF-I mRNA was decreased to the same extent in kidneys of both octreotide-treated and untreated diabetic rats. In addition, IGF-IR gene expression was unchanged by the short duration of the diabetic state and was unaffected by octreotide treatment. Thus, the prevention of the increase in renal IGF-I by octreotide in diabetes cannot be related to effects on kidney IGF-I or IGF-IR gene expression.

Concomitantly, with the increase of endogenous kidney IGF-I in early STZ diabetes, an increase in renal IGFBP-1 mRNA and protein was seen, while IGFBP-3 mRNA and protein were found to be slightly increased or unchanged (6–8), thereby giving evidence that the rise in renal IGF-I may be attributed to the upregulation of IGFBP-1 and IGFBP-3 gene expression and proteins. This finding corroborates the finding that IGF-I binds to low molecular weight IGFBPs in the diabetic kidney (8). IGFBP-5 mRNA tends to decrease in the cortex and increase in the medulla of the diabetic rat during the early phase of diabetes (6). In our study, untreated diabetes for up to 7 days resulted in a twofold increase in IGFBP-1 mRNA in the whole kidney. A similar increase in IGFBP-1 mRNA was seen when measured separately in the cortex and medulla. Treatment with octreotide prevented the early increase (2 days after STZ injection) of IGFBP-1 mRNA in the whole kidney and had a similar effect on the cortex. However, after 7 days of diabetes, octreotide failed to prevent the increase in renal IGFBP-1 mRNA. We did not demonstrate significant change in renal IGFBP-3 and IGFBP-5 gene expression after 2 and 7 days of diabetes whether treated or

TABLE 4
Kidney 30-kDa IGFBPs, IGFBP-3, and IGFBP-4 in nondiabetic control, untreated diabetic, and octreotide-treated diabetic animals

| | Day 2 | | | Day 7 | | |
|---|---|---|---|---|---|---|
| | Control | Diabetes | Diabetes + octreotide | Control | Diabetes | Diabetes + octreotide |
| *n* | 6 | 6 | 6 | 6 | 6 | 6 |
| 30-kDa IGFBPs (AU/mm$^2$) | 133 ± 10 | 255 ± 40* | 121 ± 27‡ | 119 ± 3 | 215 ± 16* | 206 ± 19† |
| IGFBP-3 (AU/mm$^2$) | 11.4 ± 1.8 | 21.9 ± 6.0 | 9.7 ± 2.5 | 10.5 ± 0.7 | 11 ± 1.7 | 9 ± 1.9 |
| IGFBP-4 (AU/mm$^2$) | 17.6 ± 4.3 | 9.8 ± 2.4 | 6.6 ± 1.4† | 16.8 ± 2.9 | 7.6 ± 1.4* | 4.9 ± 0.8† |

Data are means ± SE. The numbers are relative density of the bands as measured by Western ligand blotting. *Control versus diabetes; †control versus diabetes + octreotide; ‡diabetes versus diabetes + octreotide. $P < 0.05$.

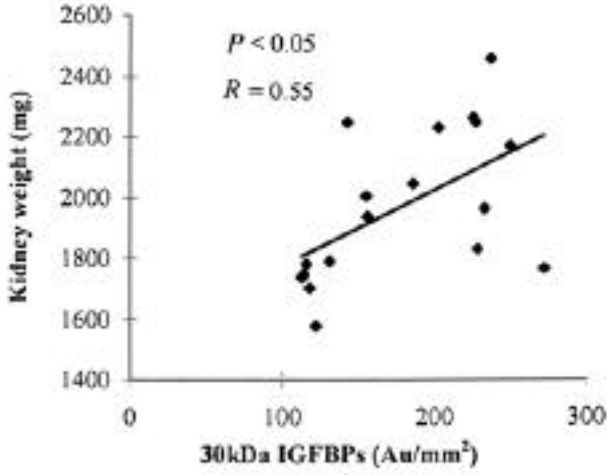


**FIG. 6. Correlation between 30-kDa IGFBPs and kidney weight in control rats, diabetic rats, and diabetic rats treated with octreotide 7 days after the induction of diabetes.**

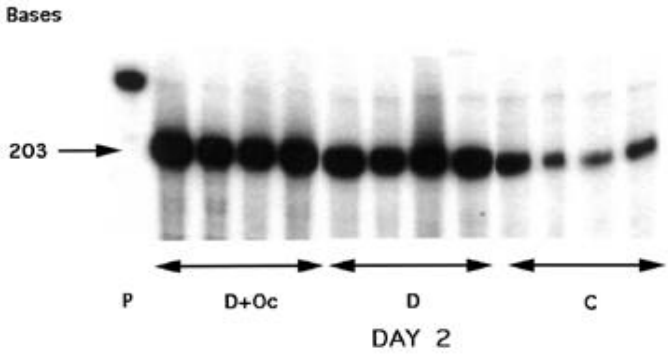


**FIG. 7. Expression of IGFBP-1 mRNA in livers of nondiabetic controls (C), untreated diabetic (D), and octreotide-treated diabetic animals (D + Oc) on day 2 after induction of diabetes. Levels of IGFBP-1 mRNA were measured by solution hybridization RNase protection assays as described in METHODS. Autoradiograph obtained from four representative animals. The arrow on the left denotes the position and size of the band corresponding to IGFBP-1 mRNA and the relative probe.**

untreated with octreotide. Other studies that demonstrated changes in renal IGFPB-3 and IGFBP-5 gene expression during the early phase of diabetes had marginal results that did not reach significance similar to our results (6–8).

The Western ligand blotting method yielded four different bands of IGFBPs with apparent molecular weight of 38–42 kDa (doublet), 30 kDa, and 24 kDa. The doublet band corresponded to IGFBP-3, the 24-kDa band to IGFBP-4, while the 30-kDa band in the kidney represents IGFBP-1, IGFBP-2, or IGFBP-5, as these have a similar molecular weight in the rat. Because of the limited amount of tissue, kidney IGFBPs were measured only in whole kidney homogenates of six animals from each group by means of Western ligand blotting.

In accordance with its effect on IGFBP-1 gene expression in whole kidney homogenates, octreotide prevented the early increase in the 30-kDa IGFBPs (containing IGFBP-1) in the kidney of day 2 diabetic rats but failed to prevent the increase in renal IGFBP-1 after 7 days of diabetes. Renal IGFBP-3 protein, however, was not changed by the diabetic state, whether treated or untreated with octreotide. Thus, this study reveals a possible novel mechanism by which octreotide may prevent the increase in kidney IGF-I and size during the very early phase of diabetes by preventing the early diabetic-related increase in renal IGFBP-1 gene expression and protein. These results can also explain why octreotide has its beneficial effect only at the very early stage of diabetes. Seven days after the induction of diabetes, octreotide no longer prevented the increase in IGFBP-1 mRNA and protein, resulting in similar renal IGF-I levels, and in parallel, similar renal growth in both octreotide-treated and untreated diabetic rats, beginning about 7 days after the induction of diabetes. This assumption is supported by the finding that after 30 days of diabetes, renal size in diabetic rats treated with octreotide for 1 week from diabetes induction was similar to that of diabetic rats treated with octreotide from diabetes induction and during the 30 days until death (unpublished observations). Renal size of both groups, however, was smaller than those of untreated diabetic rats because the main renal growth in diabetic rats occur within the first 7–10 days of diabetes at the time when the effect of octreotide is maximal (unpublished observations).

Although octreotide prevented the early increase in kidney IGFBP-1 mRNA and protein, it did not prevent the diabetes-induced early increase in liver IGFBP-1 mRNA, suggesting an organ-specific mode of action of the drug. Indeed, when normal rats were injected with octreotide, we found that within 24 h, octreotide increased the expression of liver IGFBP-1, while in the kidneys IGFBP-1 gene expression was unchanged. These findings are in line with recently published data (21,22). The fact that octreotide had influenced IGFBP-1 mRNA in the liver but not in the kidney of normal rats, while in diabetic rats kidney but not liver IGFBP-1 mRNA is influenced, could be due to the difference of insulin levels in the two situations. Thus in the presence of normal insulin levels, octreotide upregulated hepatic IGFBP-1 mRNA levels, as was also shown by others (21,22), while in the presence of low insulin levels, the increase in liver IGFBP-1 mRNA is mainly due to the hypoinsulinemic state, and a further increase by octreotide, if present, may be too small to be detected. In the

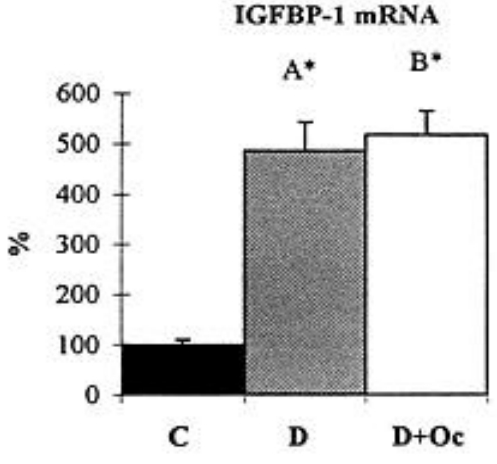


**FIG. 8. Expression of IGFBP-1 mRNA in livers of nondiabetic controls (C) ($n$ = 12), untreated diabetic (D) ($n$ = 12), and octreotide-treated diabetic animals (D + Oc) ($n$ = 13) on day 2 after induction of diabetes. Levels of mRNA were measured by solution hybridization RNase protection assays as described in METHODS. The values are the relative density of the band as percentage of control. Results are the means ± SE. A, control versus diabetes; B, control versus diabetes + octreotide. *$P$ < 0.001.**

presence of normal blood insulin levels, the influence of octreotide in downregulating the renal IGFBP-1 mRNA levels is too small to be detected. However, when renal IGFBP-1 mRNA is upregulated by the hypoinsulinemic state, the opposite effect of the drug is seen. The fact that renal IGFBP-1 gene expression and protein levels are more insulin dependent than IGFBP-3 and IGFBP-5 gene expression and protein might be the reason why in diabetes, mainly renal IGFBP-1 levels are influenced by octreotide.

The mechanism by which octreotide prevents the increase in IGFBP-1 mRNA in the kidney during hypoinsulinemic state would seem to operate through a direct action on gene regulation in the kidney since, at the same time, the increase in IGFBP-1 mRNA in the liver is unchanged. A decrease in circulating GH or insulin levels caused by octreotide treatment could separately influence IGFBP-1 in the kidney. However, a decrease in circulating GH would, if anything, increase IGFBP-1 gene expression (23). Furthermore, serum insulin and glucose levels were similar in the octreotide-treated and untreated diabetic rats. Serum IGF-I levels, however, were lower in the octreotide-treated rats when compared with control and/or untreated diabetic rats, suggesting another possible way by which octreotide could prevent the increase in renal IGF-I levels in the diabetic rat. The fact that serum IGFBP-3 was suppressed by octreotide in the diabetic rats in accordance with low GH levels suggests that the decrease in serum IGF-I seen in the octreotide-treated rats in part was the result of GH suppression (24).

In summary, the octreotide-induced prevention of increased renal IGF-I and size in the early stage of diabetes may be explained by the capacity of the drug to prevent the increase in renal IGFBP-1 mRNA and protein in the diabetic kidney. However, other mechanisms may exist. Further studies are required to explore the influence of octreotide on other growth factors including parts of the IGF system that are known to be altered in early diabetes (6,25,26).

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