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The accumulation of IGF-I in kidneys of streptozotocin-diabetic adult rats is not associated with elevated plasma GH or IGF-I levels

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Nephropathy is a major complication of diabetes mellitus and is associated with expansion of the mesangium and an increase in kidney size in both humans and rats. Interestingly, early kidney enlargement occurs only in postpubertal animals, and is preceded by a significant increase in the levels of extractable renal IGF-I. This study examined the possibility that this difference is GH dependent, and that very early changes in plasma GH and/or IGF-I in the adult animal are associated with an early accumulation of renal IGF-I. Silastic jugular catheters were placed in adult (13-14 week) male Sprague-Dawley (S-D) rats for blood collection and drug injection. Serial blood samples were taken every 30 min in groups of saline control and streptozotocin (STZ) (50 μg/kg, IV) rats from 1-6 h, 9-15 h, and 24-30 h post-injection, and plasma GH profiles were determined by RIA. Renal IGF-I content was assessed following acid extraction. Following STZ, there was an immediate, step-wise reduction in peak GH levels (saline controls, 54 ± 7 ng/ml *vs* 30 ± 5 (1-6 h); 23 ± 10 (9-15 h); and 13 ± 3 ng/ml (24-30 h post-STZ); $P < 0.05$ for all STZ groups *vs* control). The same significant step-wise reduction was observed in the integrated area under the curve for GH. A separate group of rats were treated with a GH-releasing factor antagonist (GRF-AN) for 5 days prior to STZ, to suppress pulsatile GH release, and reduce plasma IGF-I. Chronic GRF-AN administration reduced plasma IGF-I levels significantly to 63% of control values ($P < 0.01$). However, despite the reduction in plasma IGF-I, renal IGF-I remained significantly elevated 24 h post-STZ compared with controls and not significantly different from animals treated with STZ alone (467 ± 49 ng IGF-I/g KW in control saline *vs* 778 ± 100 in saline/STZ and 705 ± 87 ng IGF-I/g KW in chronic GRF-AN/STZ rats ($P < 0.05$)). In conclusion, following STZ administration in the adult rat, there is an immediate reduction in GH levels, indicating the renal IGF-I accumulation occurs without initial increases in plasma GH levels. Furthermore, when plasma IGF-I levels in the adult are significantly reduced renal IGF-I content remains elevated. These data suggest that early diabetic renal growth is not associated with elevated circulating GH levels, and that high basal plasma IGF-I levels are not necessary for IGF-I accumulation.

Keywords: diabetes; kidney; IGF-I; growth hormone; rat

Introduction

Nephropathy is a major complication of type I (insulin-dependent) diabetes, and is associated with the kidney enlargement which occurs in adult humans and streptozotocin (STZ)-induced diabetes in postpubertal rats (Ross & Goldman, 1971; Wiseman *et al.*, 1985). In the STZ animal model of diabetes, kidney weight is increased rapidly, and is significantly higher than baseline values as early as 2 days after injection of STZ (Bach & Jerums, 1990). Several lines of evidence suggest a role for the GH/IGF-I axis in diabetic renal growth. It has been shown that diabetic renal hypertrophy in postpubertal rats is preceded by a rise in extractable IGF-I in the kidney (Phillip *et al.*, 1994), which reaches a peak 24 (Bach & Jerums, 1990) to 48 (Flyvbjerg *et al.*, 1990) h after the induction of diabetes. This rapid increased in renal IGF-I occurs without elevations in renal IGF-I or IGF-I receptor mRNA, but with significant increases in renal IGFBP-1 (Phillip *et al.*, 1994). Interestingly, prepubertal diabetic rats do not exhibit an increase in kidney IGF-I, or kidney size. These observations suggest that one of the hormones that increases during puberty (such as testosterone, GH, or IGF-I) may affect kidney growth in diabetes. Castration has no effect on diabetic kidney growth in mice (Broulik & Schreiber, 1982) and rats (Bach & Rechler, 1992), suggesting that testosterone does not play a direct role in these paradigms.

In hypersomatotropism, which is associated with elevated serum IGF-I levels, glomerular filtration rate, kidney IGF-I levels, and kidney weight increase (O'Shea & Tayish, 1992). Administration of octreotide, a somatostatin analogue, prevents the increase in kidney size and tissue IGF-I without affecting serum glucose levels (Flyvbjerg *et al.*, 1989). Furthermore, diabetes-related kidney changes are attenuated in postpubertal GH-deficient dwarf rats compared with intact rats (Bach *et al.*, 1991). Studies by Tannenbaum (1981) determined that plasma GH peaks begin to diminish 18-24 h after STZ administration, however earlier time points were not assessed. Recently, in another model of renal growth, the mechanisms initiating compensatory renal growth following uninephrectomy have been shown to be different in pre- and postpubertal rats (Haramati *et al.*, Mulroney *et al.*, 1992(1); Mulroney *et al.*, 1992(2)). Indeed, adult uninephrectomized male rats have an early, transient increase in pulsatile GH release, which is absent in the juvenile animal. In addition, suppression of GH release in the adult animal attenuates renal growth post-uninephrectomy, indicating that the growth is dependent on this early change in GH secretion. Therefore, it is possible that a rapid, transient increase in GH may also play a role in initiating IGF-I retention in the adult STZ-diabetic kidney. Since differences in plasma IGF-I levels with age may be responsible for the alternate renal outcomes following administration of STZ, reducing the adult plasma IGF-I levels prior to STZ administration would help clarify this possibility. In the present study we determined: 1) if there are early transient increased in circulating GH that could stimulate renal IGF-I accumulation; and 2) whether reducing plasma IGF-I levels in adult rats would reduce the STZ-induced increase in renal IGF-I.

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Methods

General surgical procedure

Adult (12–13 week) male Sprague-Dawley rats (Harlan) weighing 430 ± 18 g were anesthetized with sodium pentobarbital (20 mg/kg) and Silastic catheters were placed in the right jugular veins. The animals were placed in one of 3 experimental groups 24 h later, after an overnight fast. In all groups, plasma samples were obtained to determine glucose and IGF-I concentrations.

Growth hormone profiles following STZ

To assess whether an immediate, transient increase in GH, contributes to the accumulation of IGF-I in STZ-diabetic kidneys, plasma GH levels were determined in conscious, freely moving animals following saline or STZ injection. The jugular catheter was attached by a 23-gauge metal tube to a length of PE-50 tubing, which allowed access to blood sampling and drug injection outside the cage without disturbing the animals. The animals were injected IV at 0830 h with either saline or STZ (50 mg/kg, in 0.1 ml) and blood samples (0.15 ml) were obtained every 20 min over a 30 h period in 3 separate groups of animals: 1–6 h post-STZ or saline ($n = 7$), 9–15 h ($n = 6$), and 24–30 h ($n = 6$). Plasma was extracted and packed blood cells were reinjected to minimize blood losses. At the end of the experimental period, the animals were decapitated and kidneys were removed, flash-frozen in liquid nitrogen, and stored at −70°C until assay. One kidney from each control and STZ-treated animal was used to determine renal IGF-I concentrations, and total RNA was extracted from the other kidney to assess IGF-I and IGF binding protein (BP)-I gene expression.

Effect of pulsatile GH suppression on renal IGF-I accumulation

To determine if suppression of GH secretion inhibits the accumulation of renal IGF-I 24 h post-STZ, animals were injected IV with a synthetic GH-releasing factor antagonist (GRF-AN: [N-Ac-tyrl-arg2]-GRF-(1-29)-NH2) (Bachem, Torrence, CA) which competed for the GRF binding sites at the anterior pituitary. This specific antagonist has been shown to suppress the pulsatile release of GH in adult and juvenile rats without affecting basal levels of GH (Lumpkin *et al.*, 1989; Mulroney *et al.*, 1989; Mulroney *et al.*, 1992(1)). The antagonist was administered either acutely (100 μg/kg, in 0.1 ml saline), 30 min prior to STZ injection, or chronically (100 μg/kg, twice daily at 0800 and 1330 h) over either 24 h or 5 days prior to STZ injection, to reduce plasma IGF-I concentrations prior to STZ injection. GH profiles were determined in each group of GH-suppressed animals 1–6 h post-STZ ($n = 7$), as previously described, to insure the reduction in plasma GH levels. Following blood sampling, animals were decapitated, and the kidneys were weighed and frozen.

Analysis

Plasma analysis

Plasma GH concentrations were determined by double-antibody RIA using a kit provided by the National Hormone and Pituitary Program (NIH, NIDDK). The sensitivity of the RIA is 0.26 ng/ml, with intra- and interassay coefficients of variability of <7%. All samples were measured in duplicate. Peak GH levels were determined using the PC Pulsar program (Urbana, IL), and average peak levels of GH were compared from each saline and STZ group. The integrated area under the 6 h curve was determined using KaleidaGraph Software.

Plasma insulin levels were measured using a double-antibody RIA (Incstar Corp, Stillwater, MN), and plasma glucose was determined with a Beckman glucose analyser.

IGF-I concentrations were measured in acid-ethanol extracted plasma by RIA (Nichols, San Juan Capistrano, CA). This extraction technique involves the separation of IGF-I from its binding proteins by precipitation with 87.5% ethanol and 12.5% 2 M HCl (Daughaday *et al.*, 1980).

Kidney IGF-I protein

Kidney IGF-I levels were determined by homogenizing the frozen tissue in 0.1 M acetic acid with a Sybron polytron (Brinkman, Westbury, NY), and neutralizing the extract with 5 M NaOH. The extract was then placed in the RIA as described above.

RNA extraction

Total RNA was extracted from frozen tissues using the guanidinium isothiocyanate/cesium chloride method of Chirgwin *et al.*, 1979. The precipitated RNA was resuspended in sterile water and quantified spectrophotometrically at 260 nm. The integrity of the RNA was assessed on a 1.25% agarose/2.2 M formaldehyde gel by visualizing the ethidium bromide-stained 18S and 28S ribosomal RNA bands.

Solution hybridization/RNase protection assays

IGF-I and IGFBP-1 gene expression was determined in kidneys from control and STZ-injected animals using a method previously described (Lowe *et al.*, 1986, 1987; Werner *et al.*, 1989). Briefly, antisense RNA probes to IGF-I, IGF-I receptor (R), or IGFBP-1 (Phillip *et al.*, 1994) were hybridized overnight with 20 μg samples of total RNA. The hybrids were extracted with phenol:chloroform, precipitated with ethanol, and electrophoresed on a polyacrilamide/8 M urea denaturing gel. The autoradiographs were quanitified by scanning densitometry.

Statistical analysis

The statistical significance between experimental groups was evaluated by analysis of variance, followed by Duncan's multiple range test with P set at 0.05. (Luginhuhl & Schlotzhauer, 1985). Differences between two groups was determined by Student's t-tests (specifically indicated in text).

Results

Growth hormone secretion following STZ treatment

Figure 1A–D illustrates representative GH profiles from individual animals at time points following saline or STZ administration. There was an immediate, step-wise reduction in pulsatile GH secretion following STZ administration compared with saline-treated animals. Average peaks and area under the curve for GH were consistent for all control groups at the different time points, so a total for all control values was used for comparisons. Figure 2A illustrates the average peak GH levels in saline controls ($n = 18$), and animals 1–6, 9–15, and 24–30 h post-STZ. Following STZ, there was an immediate, significant reduction in peak GH levels, which continued to decline through 24–30 h, without affecting average trough baseline levels (4.8 ± 0.5 *vs* 6.5 ± 1.2 ng/ml in controls, n.s.). The reduction in peak GH levels also reduced the integrated area under the curve in the same step-wise manner (Figure 2B).

Effect of pulsatile GH suppression on renal IGF-I accumulation

Figure 3 illustrates peak GH levels in GRF-AN/STZ-treated animals compared with saline control and saline/STZ-treated animals 1–6 h after STZ or saline injection. Acute (30 min

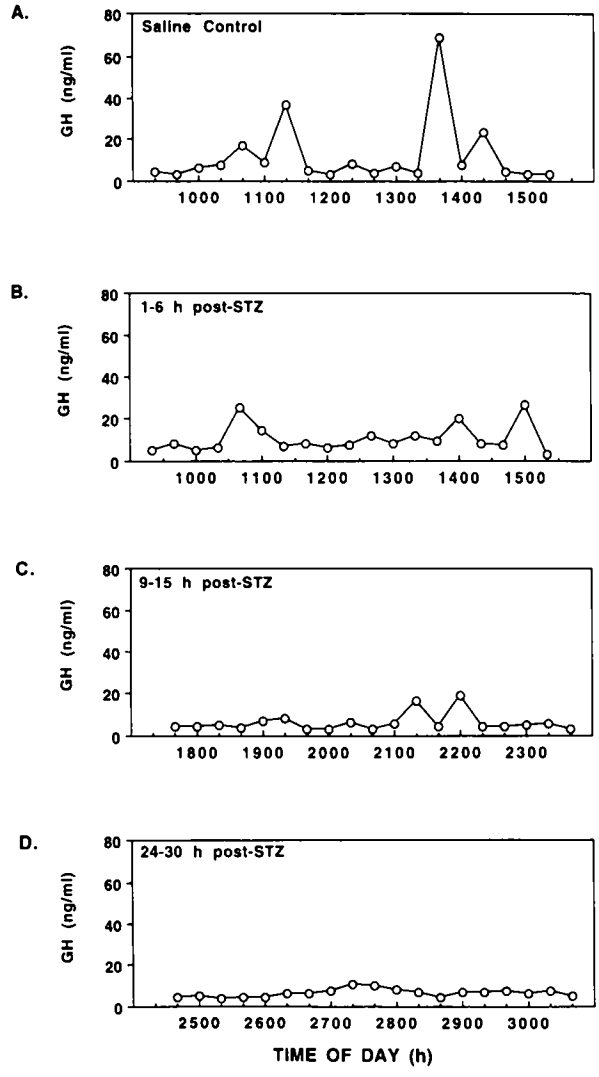

Figure 1 Representative growth hormone (GH) profiles from individual animals at time points after administration of saline or STZ. (**A**) GH profile in saline control animal, illustrating the peak GH levels compared with GH profiles after STZ injection: (**B**) 1–6 h post-STZ; (**C**) 9–15 h post-STZ; and (**D**) 24–30 h post-STZ

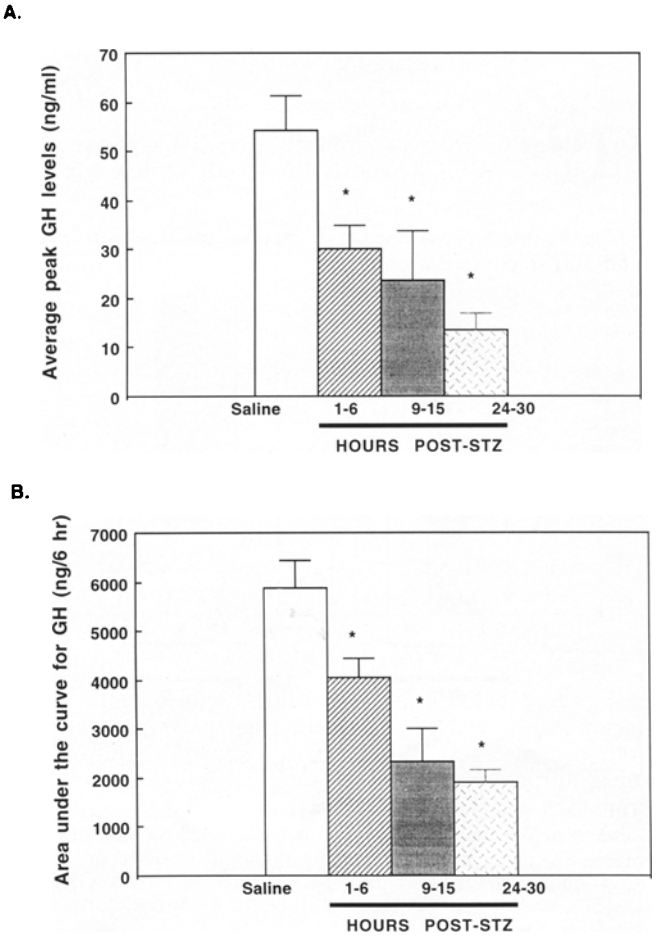

Figure 2 Average peak circulating GH levels and integrated area under the curve in adult rats following saline or STZ intravenous injection. (**A**) Peak GH levels were significantly attenuated within 6 h following STZ and continued to decrease in a stepwise fashion through 30 h post-injection. (**B**) Peak areas of GH was also significantly attenuated within 6 h following STZ and continued to decrease in a step-wise fashion through 30 h post-injection. This was a result of reductions in peak levels, without changes in basal (trough) GH levels. * = $P<0.05$ *vs* saline control

before STZ) injection of the GRF-AN significantly attenuated GH peaks and the area under the curve (1731 ± 351 ng/6 h) compared with saline or STZ controls. Chronic (5-day) treatment of animals with the GRF-AN further reduced average GH peak levels to 7.8 ± 1.4 ng/ml, with an area under the curve of 1052 ± 36 ng/6 h, which was significantly lower than that of saline controls, STZ-treated animals, or acute GRF-AN treatment ($P<0.05$). This is consistent with previous reports using this antagonist (Haramati *et al.*, 1994; Lumpkin *et al.*, 1989). Plasma IGF-I levels in control animals were comparable with previous reports (Bach & Jerums, 1990; Flyvbjerg *et al.*, 1989; Handelsman *et al.*, 1987). Twenty-four hours after administration of STZ, plasma glucose levels were significantly elevated over controls ($P<0.05$) (Figure 4). In addition, plasma insulin levels were not different between STZ and GRF-AN/STZ animals (64.3 ± 1.3 *vs* 67.8 ± 3.8 pmol/L, respectively). The chronic (5 days prior to STZ), but not acute (24 h prior to STZ) GRF-AN treatment was successful in significantly reducing plasma IGF-I concentrations to 63% and 61% of that in saline control and saline/STZ animals respectively ($P<0.05$), without changes in plasma glucose (Figure 4). As previously observed, kidney IGF-I levels were significantly elevated 24–30 h post-STZ administration. However, despite the significant attenuation in plasma IGF-I levels prior to STZ administration, there was no reduction in

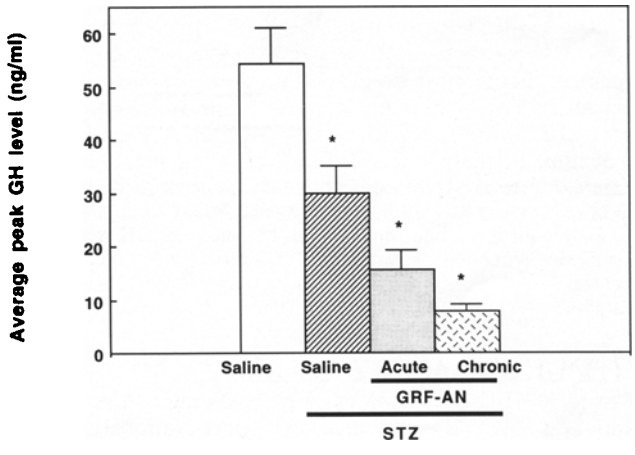

Figure 3 Average peak GH levels in the presence and absence of the GRF-antagonist and STZ. Average peak GH levels were obtained from GH profiles taken 1–6 h after saline or STZ administration. Both acute (30 min prior to STZ) and chronic (5 days prior to STZ) administration of the GRF-AN significantly suppressed pulsatile GH release compared to untreated saline controls. Indeed, chronic GRF-AN treatment was effective in abolishing GH peaks (significantly lower than STZ alone, $P<0.05$) at this early time period following STZ administration. * = $P<0.05$ *vs* saline control

kidney IGF-I in the chronic GRF-AN/STZ animals (Figure 5).

Renal IGF gene expression

To evaluate the effect of chronic (5 day) GH suppression on renal IGF-1, IGF-I R, and IGFBP-1 mRNA levels in STZ animals, RNase protection assays were performed with RNA from control and chronic GRF-AN/STZ kidneys. As observed previously in post-pubertal STZ-treated animals (Bach & Jerums, 1990), IGF-I and IGF-IR mRNA levels did not increase, and suppression of GH with the GRF-AN did not alter the response. Compared with saline controls, treatment of rats with STZ significantly increased renal IGFBP-1 mRNA by ~7-fold in both saline/STZ and chronic GRF-AN/STZ rats, despite the reduction in plasma IGF-I in the chronic GRF-AN/STZ group. Figure 6 illustrates that there was no difference in IGFBP-1 mRNA between the STZ and GRF-AN/STZ kidneys.

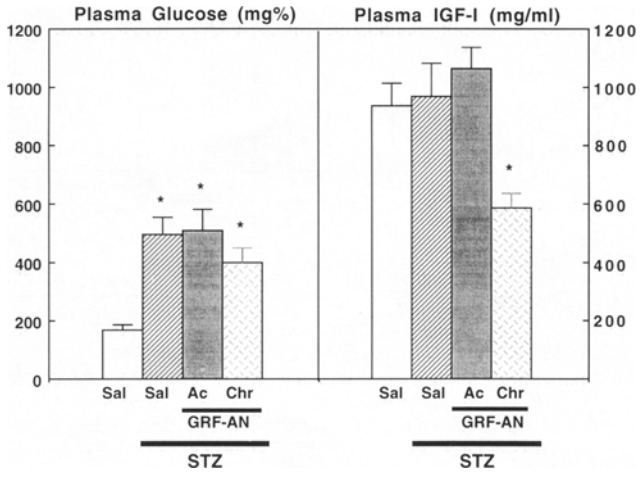

Figure 4 Plasma glucose and IGF-I levels in control and experimental animals. Twenty-four hours following STZ administration plasma glucose levels (left panel) were significantly elevated compared with saline controls: GRF-AN treatment did not affect the increase in glucose. IGF-I levels were not affected by STZ administration alone, or in combination with an acute injection of the GRF-AN, however, chronic (5 days prior to STZ) GRF-AN significantly reduced plasma IGF-I levels to ~60% of control values. * = $P<0.05$ *vs* control

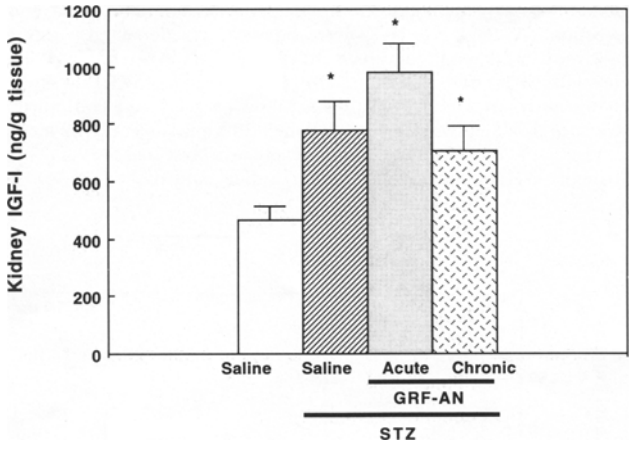

Figure 5 Renal IGF-I content in the presence and absence of GRF-AN. There was no difference in renal IGF-I accumulation after STZ treatment despite suppression of pulsatile GH release and significant reduction of plasma IGF-I levels. * = $P<0.05$ *vs* saline control

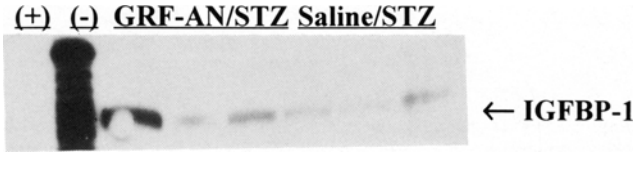

Figure 6 Representative autoradiograph of renal IGFBP-1 mRNA levels in STZ-diabetic rats in the presence and absence of GRF-AN. There was no significant difference in BP-1 gene expression in kidneys from animals that were chronically GH-suppressed (5 days). Lane 1, (+) probe with RNase; lane 2, (−) probe without RNase; lanes 3–5, GRF-AN/STZ; lanes 6–8, Saline/STZ

Discussion

Diabetes is associated with renal hypertrophy (Christiansen *et al.*, 1981; Morgensen & Andersen, 1983; Seyer-Hansen, 1976, 1983; Seyer-Hansen *et al.*, 1980), and a rapid accumulation of IGF-I in the kidneys (by 24 h) that precedes, and is thought to play a role in renal enlargement in adult, but not juvenile animals (Ross & Goldman, 1971; Wiseman *et al.*, 1985). Although IGF-I protein is elevated in the adult diabetic kidney, there is no increase in IGF-I gene expression. However, the levels of IGFBP-1 mRNA, which encodes the major kidney IGFBP, are elevated (Phillip *et al.*, 1994), suggesting that the IGF-I may be sequestered, or collected (by reduced degradation) in the kidney, as opposed to being newly synthesized. Furthermore, the age-related differences in renal enlargement and IGF-I are thought to result from the different levels of circulating IGF-I present in young and adult animals (Bach & Rechler, 1992). The present study evaluated the possible influence of plasma GH and IGF-I on elevated IGF-I levels in STZ-diabetic kidneys.

Although it is known that GH levels are attenuated in the rat following STZ administration (Bach *et al.*, 1991), the early (prior to 18 h post-STZ) changes in this growth factor are not documented, and there is the possibility that an early, transient elevation in GH secretion could stimulate the increase in renal IGF-I either by local production or via circulating IGF-I. Our findings indicate that there was no elevation in plasma GH levels, but rather an immediate, step-wise reduction in pulsatile GH release following administration of STZ to adult fasted rats, which confirms and extends the previous work of Tannenbaum. Whether this reduction in GH results directly from effects on hypothalamic GRF or pituitary GH secretion, or indirectly through rapid hyperglycemia, is not known. However, since diabetes in the human adult is associated with an increase in plasma GH levels it is possible that the effects of STZ on GH are not directly related to the induction of diabetes and subsequent renal changes.

To address whether renal IGF-I accumulation in the adult results from elevated plasma IGF-I levels compared with the juvenile, we reduced circulating IGF-I in the adult animal to ~60% of control values through chronic (5 day) administration of a specific GRF-antagonist, which suppressed the pulsatile release of pituitary GH prior to STZ administration. Despite the significant reduction in plasma IGF-I levels, renal IGF-I was still elevated, and was not different from saline/STZ animals. Also, renal IGFBP-1 mRNA was still significantly elevated, and no changes in renal IGF-I or IGF-IR gene expression was seen when plasma IGF-I was reduced. Therefore, the plasma levels of IGF-I observed in the adult animal, though reduced, may still contribute to the accumulation of IGF-I in the STZ-treated kidney.

It has been reported that renal sequestration of local and circulating IGF-I can occur by binding to IGFBPs. Werner *et al.* (1989) determined that early STZ-diabetes in adult rats was associated with increased IGF-I binding to the IGF-I receptor, as well as to a low molecular weight material that presumably represent IGFBPs (Werner *et al.*, 1990). Subsequently, renal IGFBP levels have been shown to increase in

post-pubertal STZ-diabetic rats using ligand blot analysis (Flyvbjerg *et al.*, 1992). In the present study, IGFBP-1 mRNA was elevated by ~7-fold, in the presence or absence of pulsatile GH, and despite a significant reduction in plasma IGF-I levels following chronic GRF-AN treatment. These findings suggest that sequestration of IGF-I in diabetic kidneys of adult rats may be associated with increased binding to IGFBP-1, and independent of circulating GH pulses, or high (adult) plasma levels of IGF-I.

In conclusion, these findings suggest that the accumulation of IGF-I in kidneys of post-pubertal diabetic rats is not dependent on early increases in circulating GH. Furthermore, elevated plasma IGF-I levels in the post-pubertal animals are not required for renal IGF-I accumulation, since reduction of circulating IGF-I levels prior to STZ treatment did not prevent this accumulation.

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