Reduced insulin-like growth factor-I mRNA content in liver, adrenal glands and spinal cord of diabetic rats

D.N. Ishii¹, D.M. Guertin¹, L.R. Whalen²

¹ Department of Physiology, Colorado State University, Fort Collins, Colorado, USA

² Department of Anatomy and Neurobiology, Colorado State University, Fort Collins, Colorado, USA

Summary Circulating insulin-like growth factor I (IGF-I) concentrations are known to be reduced in experimental and clinical diabetes mellitus. The IGF-I mRNA content was measured in several tissues of rats treated with streptozotocin to determine whether a correlation with neuropathy could be found. IGF-I mRNA content was sharply reduced relative to total and poly(A)⁺ RNA in diabetic liver and adrenal glands. In contrast, histone 3.3 mRNA content was not significantly reduced relative to poly(A)⁺ RNA in liver, and alpha-tubulin mRNA content instead was increased in adrenal glands, showing that the decline in IGF-I mRNAs in these tissues was selective. In addition, spinal cord IGF-I mRNA content was significantly reduced per tissue, total RNA, and poly(A)⁺ RNA after 1 and 2 weeks

of diabetes. This was correlated with a concurrent and significant decrease in conduction velocity in both spinal cord and peripheral nerves in a separate study. The decline in liver and spinal cord IGF-I mRNA was not due to streptozotocin toxicity, because it was significantly opposed by insulin which was continuously infused beginning the day after diabetes induction. These results, when taken together with those of others, indicate that the reduction in IGF-I mRNA content may be widespread among diabetic tissues, and might contribute in part to certain syndromes of diabetes, such as neuropathy. [Diabetologia (1994) 37: 1073–1081]

Key words Insulin-like growth factor, somatomedin, diabetic neuropathy, conduction velocity.

Insulin-like growth factor I (IGF-I) is an anabolic growth mediator [1, 2] regulated by growth hormone [3, 4]. Many tissues are known to produce IGF-I protein and mRNA [4–7], with liver being the predominant source of circulating IGF-I [8]. The growth rate is reduced in diabetes [9, 10] and IGF-I mRNA content is diminished in several diabetic tissues [11–13]. The extent of this biochemical pathology in diabetes deserves further study.

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Corresponding author: Dr. D.N. Ishii, Department of Physiology, Colorado State University, Fort Collins, CO 80523 USA

Abbreviations: IGF-I, Insulin-like growth factor I; SSC is 150 mmol/l NaCl and 15 mmol/l sodium citrate; STZ, streptozotocin; AUC, area under the curve.

Circulating IGF-I concentrations remain elevated long after the growth spurt [14], suggesting that, in addition to growth, IGF-I may serve to regulate other functions in mature vertebrates. Among such functions, emerging data suggest insulin and IGFs may play a prominent neurophysiological role, particularly in the development and maintenance of the neural circuitry [15]. This, in turn, has led to the hypothesis that a decline in IGF activity contributes to the pathogenesis of diabetic neuropathy [16]. This theory prompted Carsten et al. [17] to predict and find functional neuropathy in the spinal cord in an insulin ablation experiment. Fairly extensive observations now support the hypothesis that insulin and IGF-I may act together on neurons (see Discussion). It is possible, then, that the activity of IGF-I, as well as insulin, is important for support of neurons in diabetes.

The purpose of this study was to (a) add to the emerging evidence showing that disturbance in IGF-

I mRNA content is widespread among diabetic tissues, and (b) test whether there is a decline in IGF-I mRNA content in diabetic liver and spinal cord sufficiently early to implicate a role for IGF-I in the development of complications in the nervous system.

Materials and methods

Materials. Oligo(dT)₁₈ was obtained from Pharmacia (Piscataway, N.J., USA) and T4 polynucleotide kinase from Bethesda Research Laboratories (Gaithersburg, M.d., USA). Streptozotocin (STZ), porcine insulin, and glucose diagnostic kit 510 were purchased from Sigma Chemical Co. (St. Louis, Mo., USA). Mini-osmotic pump Model 2ML1 was from the ALZA Corp. (Palo Alto, Calif., USA). Sprague-Dawley rats were obtained from Charles River Laboratories, Inc. (Wilmington, Mass., USA) or Harlan (Indianapolis, Ind., USA).

The human cDNA clone pK α 1 contains the entire α -tubulin coding region plus 67 and 173 base pairs of the 5' and 3' untranslated regions, respectively [18]. The clone pMH921 is a genomic 2.6 kilobase insert containing the complete coding region of the mouse histone 3.3 gene [19, 20]. The IGF-I cDNA clone in pUC9 was a gift from Dr. A. Efstratiadis and contains the entire rat pre-pro-IGF-I coding sequence plus 340 nucleotides of 5', and 69 nucleotides of 3', non-coding sequence. This clone begins with nucleotide 1780 of exon 1, contains no nucleotides from exon 4, and ends in nucleotide 718 of exon 5 of the sequence published by Shimatsu and Rotwein [21].

Experimental procedure. All studies involving rats were done in accordance with the principles of the NIH Guide for the Care and Use of Laboratory Animals. Rats were raised on 20 g per day standard rat chow until the study, and chow and water were provided ad libitum thereafter. Male rats (10 or 12-week-old) were randomly assorted into treatment groups, fasted overnight and anaesthetized with 80 mg/kg ketamine and 0.3 mg/kg acepromazine, i.p. Diabetes was induced in 12-week-old rats with i.v. 40–50 mg/kg STZ. STZ lots can have slightly different activity. Control rats were injected with an equivalent volume (1 ml/kg) of vehicle (10 mmol/l sodium citrate, 0.9 % sodium chloride, pH 4.5). Plasma glucose concentrations were determined the following day using a glucose diagnostic kit. Only STZ-treated rats with greater than 22 mmol/l (400 mg/dl) glucose in plasma were used in this study, except in the insulin infusion study for reasons discussed.

Miniosmotic pumps were implanted s.c. in the midback of some groups the day after STZ administration. The conditions were similar to those described to maintain normoglycaemia during constant insulin infusion [22]. The pumps were filled with either vehicle (1.6 % glycerin, 7 mg/ml glutamic acid, 0.2 % phenol, pH 3.3), or vehicle containing porcine insulin. The solutions were sterilized by passage through 0.2- μ m filters. Insulin concentrations were adjusted to deliver 58.5 nmol (9.75 units) per kg body weight per day, and filled pumps were soaked in saline at 37 °C for 2 h prior to implantation in order to decrease the time lag in release of solutions.

Preparation of RNA. Following killing with carbon dioxide, total RNA was immediately extracted from various tissues by the guanidinium thiocyanate procedure of Chomczynski and Sacchi [23]; LiCl was used to remove glycogen from liver samples [24]. RNA concentration and purity were estimated by A 260 and 280 nm.

RNA electrophoresis and transfer to nitrocellulose. Equivalent amounts of RNA (40 μ g determined from absorbance measurements) were electrophoresed through 0.8 % agarose gels containing formaldehyde, as described [25]. Ethidium bromide staining determined the position of 18S and 28S rRNAs and confirmed that equivalent amounts of undegraded RNA had been loaded. The RNA was transferred to nitrocellulose [26]. Alternatively, samples were heat-treated in formaldehyde, rapidly cooled on ice, and directly loaded (8 μ g in duplicate) onto high capacity BA85 nitrocellulose using a slot blot filtration device (Schleicher and Schuell, Keene, N.H., USA) similar to that described for dot blots [27]. RNA retention was linear up to at least 10 μ g/slot.

Hybridization to RNA. Nitrocellulose blots were hybridized to nick-translated [28] ³²P-labelled DNA probes for 16–18 h at 42 °C. Single-stranded DNA probes were labelled to high specific activity using Taq primer extension [29] to study spinal cord RNA. Blots were washed for 45–60 min each with 2 $\times$ wash buffer at 42 °C, 2 $\times$ wash buffer at 68 °C, twice with 1 $\times$ wash buffer at 68 °C, and 0.5 $\times$ wash buffer at 68 °C. Single-strength wash buffer (denoted as 1 $\times$) contained 150 mmol/l NaCl, 15 mmol/l sodium citrate, 10 mmol/l sodium phosphate, 0.03 % pyrophosphate, and 0.05 % sodium dodecylsulphate, pH 7.0.

The procedure of Harley [30] was followed to end-label oligo(dT)₁₈. This reagent was used to measure poly(A)⁺ RNA. The slot blot filters were pre-soaked for 5 min in 5 $\times$ SSC (1 $\times$ SSC is 150 mmol/l NaCl, 15 mmol/l sodium citrate) and then hybridized for 1 h at 30 °C with ³²P-labelled oligo(dT)₁₈ (1.02 nmol) in an 8.0 ml solution containing 5 $\times$ SSC, 5 $\times$ Denhardtts, 10 mmol/l Na₂HPO₄, 1 mmol/l Na₂H₂P₂O₇, and 0.125 % sodium dodecylsulphate. The filters were then washed three times for 5 min in 2 $\times$ SSC at 30 °C.

Boiling twice for 20 min in water was sufficient to remove all hybridized cDNA, as indicated by autoradiography, and some nitrocellulose blots were rehybridized to various cDNA or oligonucleotide probes.

Autoradiography and densitometry. Autoradiograms were prepared on X-ray film using Cronex Lightning Plus enhancing screens at –70 °C, scanned on a GS 300 Scanning Densitometer connected to a computer, and data were analysed with the GS360 software package (Hoefer Scientific, San Francisco, Calif., USA). The best fit Gaussian curves were used to determine area under curves (AUC), which are reported in arbitrary units in the Figures and Tables. IGF-I mRNA per total RNA was calculated by dividing the IGF-I mRNA AUC values by total RNA loaded per sample, poly(A)⁺ RNA per total RNA was calculated by dividing the oligo(dT) AUC values by total RNA loaded per sample, and IGF-I mRNA per poly(A)⁺ RNA was calculated by dividing the former by the latter. The linear range for measurements was determined by including a concentration series of RNA in blots, and by taking several autoradiographic exposures of different durations.

Statistical analysis

Statistical calculations were made with Student's *t*-test. Means and SEM are shown.

Results

IGF-I mRNA content in liver. Rats (12 weeks old) were randomly assigned to two groups, one of which was treated with STZ to induce diabetes. After 8 days, RNA was isolated from the livers of both groups. Equivalent amounts of total RNA were analysed on blots to detect IGF-I mRNAs, poly(A)⁺ RNA and histone 3.3 mRNA (Table 1). IGF-I mRNA content was significantly reduced in diabetic liver by about 93 % whether measured relative to total RNA or poly(A)⁺ RNA. In contrast, histone 3.3 mRNA content was reduced to a lesser degree per total RNA, but this difference was not selective because it was not reduced per poly(A)⁺ RNA. The relative mRNA pool size, estimated by the oligo(dT) hybridization, did not change appreciably. The results show that the sharp decline in IGF-I mRNA content in diabetic liver was not shared by the total mRNA pool.

Kinetics of IGF-I mRNA decline in liver. The rate of loss of IGF-I mRNA may provide clues as to the mechanism, as well as relationship to the decline in circulating IGF-I. The rate at which IGF-I mRNA was lost in RNA from diabetic liver is shown in Figure 1. There was no detectable change in the IGF-I mRNA content on the first day following diabetes induction. Thereafter, there was a 55 % decrease in IGF-I mRNA content between 1 and 2 days, no further change between 2 and 3 days, and a second decline of about 40 % between 3 and 7 days. A spontaneous trend towards recovery between 1 and 10 weeks was seen, but only two animals were assayed at 10 weeks making it difficult to attribute significance to this observation.

Insulin infusion opposes the decline in liver IGF-I mRNA. The possibility that IGF-I mRNA levels might decline as a result of STZ toxicity to liver cells producing this transcript was investigated (Table 2). After STZ administration, rats had elevated plasma glucose concentrations at 20 h and were randomly assigned to two treatment groups.

Miniosmotic pumps were implanted subcutaneously 20 h post-treatment, and released either vehicle or insulin at a constant rate in a similar manner to that previously shown to maintain normoglycaemia [22]. RNA was extracted from livers of the two groups of diabetic rats 7 days after STZ administration. RNA was also isolated from a third group consisting of age-matched non-diabetic animals. The IGF-I mRNA levels were much lower in the diabetic rats treated with vehicle relative to untreated, non-diabetic rats. However, the IGF-I mRNA content remained significantly higher in the insulin-treated vs the vehicle-treated diabetic group, but did not quite return IGF-I mRNA content to normal despite attainment of nearly normal glucose concentrations.

Table 1. IGF-I mRNA and histone 3.3 mRNA content, relative to total RNA and poly(A)⁺ RNA, in diabetic rat liver

Assay	Non-diabetic (%) ^a	Diabetic (%)
IGF-I mRNA		
per total RNA	100 ± 11	7.1 ± 2.1 ^b
per poly(A) ⁺ RNA	100 ± 15	7.3 ± 2.1 ^b
Histone 3.3 mRNA		
per total RNA	100 ± 7	67 ± 15 ^c
per poly(A) ⁺ RNA	100 ± 16	77 ± 15

Rats (12-weeks-old) were injected either with STZ to induce diabetes or an equal volume of vehicle. Eight days later, total RNA was isolated from livers, and analysed (8 µg) on a slot blot. The filter was hybridized in succession to ³²P-labelled clone pMH921 and oligo(dT) in order to detect histone 3.3 mRNA and poly(A)⁺ RNA, respectively. Autoradiograms of both this slot blot, and a Northern blot detecting IGF-I mRNA, were scanned on a densitometer, and a computerized program was used to measure the relative area under each curve (AUC).

^a The relative AUC values are means ± SEM (*n* = samples from five rats run in duplicate), and are percentages relative to non-diabetic values.

^b *p* < 0.005 between samples from diabetic and non-diabetic rats.

^c *p* < 0.05 between samples from diabetic and non-diabetic rats

Fig. 1. Effect of diabetes duration on the kinetics of decline of IGF-I mRNA content in liver. Rats (12 weeks old) were randomly assigned into two treatment groups. Groups were injected with either vehicle alone (non-diabetic), or STZ to induce diabetes. At various times thereafter, as indicated, groups of animals were assayed. RNA was isolated from liver and equivalent amounts (8 µg per slot) analysed for IGF-I mRNA content. The values are means ± SEM (number of animals in group shown next to symbol) of AUC shown as percentages relative to age-matched non-diabetic rats. Where *n* = 2 at 10 weeks, the values instead are ranges. (●), diabetic; (○), non-diabetic

These results unequivocally showed that the decline in IGF-I mRNA levels was not the result of STZ toxicity to liver cells producing this transcript. Insulin was capable of inhibiting the decline in IGF-I mRNA content, even though its infusion was not started until 1 day after the STZ treatment.

Table 2. Effect of insulin infusion on serum glucose and liver IGF-I mRNA levels in rats treated with STZ

Treatment	N	Glucose (mmol/l)		IGF-I mRNA (AUC) ^a
		20 h	1 week	
Non-diabetic	4	ND	5.6 ± 0.4	100 ± 6
Diabetic + vehicle	5	16.3 ± 2.1	31.3 ± 3.5	32 ± 3
Diabetic + insulin	5	17.2 ± 1.2	6.9 ± 1.3 ^b	56 ± 9 ^c

Rats (12 weeks old) were administered STZ and plasma glucose levels were determined after 20 h. The rats were randomly assigned to two groups, both of which were implanted s.c. with miniosmotic pumps. The pumps delivered either 58.5 nmol insulin · kg body weight⁻¹ · day⁻¹ or vehicle only. One week after the diabetes induction, plasma glucose levels were determined, and RNA was purified from liver and analysed for relative IGF-I mRNA content on a Northern blot. A third group of untreated non-diabetic animals were also included.

The rats were not fasted prior to glucose assay because of the possibility of hypoglycaemia in the group receiving continuous insulin infusion.

^a The AUC of densitometric scans is shown. The values are means ± SEM, shown as percentages relative to non-diabetic values. *n* = number of animals. ND, not determined.

^b *p* < 0.0005 between the two diabetic groups.

^c *p* < 0.025 between the two diabetic groups

Prior to removing liver, blood samples were taken. The mean plasma glucose concentration in insulin-treated diabetic rats was significantly reduced relative to the mean in vehicle-treated diabetic rats, and was slightly but not significantly elevated relative to the mean in normal animals. This slight elevation could be traced to two diabetic rats that had relatively high plasma glucose concentrations (9.9 and 10.0 mmol/l), and very low IGF-I mRNA levels, despite insulin-treatment. Whether this was due to poor pump performance resulting from occlusion or due to other causes is not known.

IGF-I mRNA content in adrenal glands. To study whether IGF-I mRNA content is reduced in other diabetic tissues, the adrenal glands were examined (Fig. 2). Animals were 10 or 12 weeks old at the onset of the study, and were assayed after 5 or 10 weeks' diabetes duration. The adrenal IGF-I mRNAs were more abundant and about the same in size as the various transcripts observed in neonatal brain and muscle, but the content was profoundly reduced in all diabetic adrenal glands (Fig. 2a). This autoradiogram was scanned on a densitometer, and there was a significant difference (*p* < 0.005) between non-diabetic and diabetic samples: 769 ± 196 (*n* = 4), non-diabetic; 99 ± 19 (*n* = 6), diabetic. There was an 87 % decline in the amount of IGF-I mRNA relative to total RNA in adrenal glands, which was comparable to the 93 % decline observed earlier in liver. When data from only the 5-week diabetic rats were considered, the IGF-I mRNA content was also significantly reduced (*p* < 0.005). The blot was boiled and rehybridized to ³²P-labelled cDNA clone pKα1 (Fig. 2b). α-Tubulin mRNA was increased, rather than decreased, relative to total RNA: 664 ± 55 (*n* = 4), non-diabetic; 948 ± 30 (*n* = 6) diabetic (*p* < 0.005).

The same adrenal RNA samples (8 µg/lane) were analysed further on slot blots by hybridization successively to ³²P-labelled IGF-I cDNA and oligo(dT) as

Fig. 2. Effect of diabetes on abundance of IGF-I and tubulin mRNAs in adrenal glands. Rats (10 and 12 weeks old) were randomly assorted into two groups, one of which was treated with STZ to induce diabetes. After 5 (lanes 2–9) or 10 (lanes 10 and 11) weeks of diabetes, RNA was isolated from adrenal glands. Equivalent amounts of RNA (40 µg) from each sample were electrophoresed in 0.8 % agarose gels containing formaldehyde. Ethidium bromide staining confirmed that equal amounts of undegraded RNA were present in each lane, and revealed the presence of 18 and 28 S rRNA bands. Following transfer to nitrocellulose, the samples were hybridized to a nick-translated cDNA containing the coding sequence of rat IGF-I. A print of the autoradiogram is shown (a). The mean AUC values were decreased in diabetic animals (*p* < 0.005). The blot was stripped and rehybridized to nick-translated cDNA clone pKα1 to detect α-tubulin mRNAs (b). Tubulin mRNA levels (mean AUC values) were elevated in diabetic animals (*p* < 0.005). C, non-diabetic control; D, diabetic; B, 2-day-old rat brain; M, 4-day-old skeletal muscle

Table 3. IGF-I mRNA content relative to total RNA and poly(A)⁺ RNA in diabetic rat adrenal glands

Treatment	<i>n</i>	IGF-I mRNA per total RNA	Poly(A) ⁺ RNA per total RNA	IGF-I mRNA per poly(A) ⁺ RNA
Non-diabetic	4	100 ± 16	100 ± 10	100 ± 7
Diabetic	6	12 ± 3 ^a	101 ± 6	13 ± 3 ^a

Samples of RNA (8 µg/lane) from the experiment shown in Figure 2 were analysed further on a slot blot. The blot was hybridized first to detect IGF-I mRNA, boiled, and rehybridized to detect poly(A)⁺ RNA using ³²P-labelled oligo(dT). The

relative AUC values in arbitrary units are means ± SEM (*n* = number of animals), shown as percentages relative to non-diabetic values.

^a *P* < 0.0005 between non-diabetic and diabetic groups

Table 4. Total RNA and IGF-I mRNA content in diabetic rat spinal cord

Treatment	<i>n</i>	RNA per cord	IGF-I mRNA		
			per cord	per RNA	per poly (A) ⁺ RNA
Non-diabetic	8	187 ± 13	100 ± 18	100 ± 15	100 ± 9
Diabetic					
8 days	4	204 ± 9	46 ± 9 ^a	51 ± 8 ^a	91 ± 6
14 days	4	206 ± 7	39 ± 5 ^b	44 ± 5 ^b	57 ± 7 ^c

Rats (12 weeks old) were randomly assigned into two groups, and injected either with vehicle or STZ. RNA was isolated from whole spinal cords of non-diabetic animals, and rats diabetic for 8 and 14 days. The total RNA per cord was determined (µg). Equivalent amounts of total RNA (8 µg/lane) were loaded on slot blots and hybridized to detect IGF-I

mRNA and poly(A)⁺ RNA. Relative AUC values are shown as means ± SEM (*n* = number of animals) in percentages relative to non-diabetic values. Diabetic and non-diabetic groups were compared.

^a *P* < 0.05; ^b *P* < 0.025; ^c *P* < 0.01

shown in Table 3. The IGF-I mRNA content was significantly decreased relative to both total RNA and poly(A)⁺ RNA in adrenal glands from diabetic rats. However, no statistical difference was found in oligo(dT) hybridization between RNA from non-diabetic and diabetic rats, showing that the poly(A)⁺ RNA pool size was unchanged relative to total RNA. The data for adrenal glands, as for liver, show that IGF-I mRNA transcripts are selectively reduced in diabetes.

IGF-I mRNA content in spinal cord. The IGF-I gene is expressed in rodent CNS [4, 6]. The profile of spinal cord IGF-I mRNAs on Northern blots was similar to that of brain. IGF-I mRNA content was reduced in diabetic cord (Fig. 3).

The content of total RNA, poly(A)⁺ RNA, and IGF-I mRNA was measured in spinal cords from rats which were 12 weeks old at the onset, had been treated with 40 mg/kg STZ, and had been diabetic for 8 and 14 days (Table 4). The IGF-I mRNA content was profoundly (50–60%) and significantly decreased per spinal cord and per total RNA in rats that had been diabetic for 8 and 14 days, and significantly decreased per poly(A)⁺ RNA at 14 days. The total RNA content per spinal cord was not significantly different between non-diabetic and diabetic rats.

Using a slightly higher STZ concentration (47 mg/kg), IGF-I mRNA content per poly(A)⁺ RNA was found to be significantly reduced (*p* < 0.005) in diabetic rats (vehicle) as early as 1 week after induction

Fig. 3. Effect of diabetes on IGF-I mRNAs in spinal cord. Rats (12 weeks old) were randomly assigned into groups. Some were non-diabetic untreated controls, and others were made diabetic with 47 mg/kg STZ. Diabetic rats were confirmed to be diabetic by glucose assay at 20 h, and implanted with miniosmotic pumps that released vehicle or insulin (58.5 nmol · kg body weight⁻¹ · day⁻¹). One week later, RNA was isolated from spinal cords and equivalent amounts (40 µg/lane) analysed on Northern blots to detect IGF-I mRNAs. Adult liver RNA (10 µg/lane) is shown for comparison. Ethidium bromide staining confirmed that equivalent amounts of RNA had been transferred to blots, and revealed the position of 18S and 28S rRNA bands. Lanes 1 and 4, non-diabetic; lane 2, insulin-treated diabetic; lane 3, vehicle-treated diabetic; and lane 5, liver

Table 5. Effect of insulin infusion on spinal cord IGF-I mRNA content in rats treated with STZ

Treatment	n	IGF-I mRNA per poly(A) ⁺ RNA	poly(A) ⁺ RNA per total RNA
Non-diabetic	3	100 ± 6	100 ± 8
Diabetic (vehicle)	4	60 ± 6 ^a	110 ± 13
Diabetic (insulin)	4	81 ± 6 ^b	106 ± 10

Rats (12 weeks old) were treated with STZ and implanted with pumps that released either insulin or vehicle, in the same experiment described in Figure 3. One week after the induction of diabetes, RNA was isolated. Slot blots with equivalent amounts of spinal cord RNA (8 µg/slot) were hybridized to detect IGF-I mRNA. Blots were stripped and rehybridized to detect poly(A)⁺ RNA. Non-diabetic rats were also studied. The relative AUC values in arbitrary units are means ± SEM (*n* = number of rats) shown as percentages relative to non-diabetic values.

^a *P* < 0.005, diabetic (vehicle) vs non-diabetic group.

^b *P* < 0.05, diabetic (insulin) vs diabetic (vehicle) group

of diabetes at 12 weeks of age (Table 5). STZ toxicity to spinal cord cells was probably not the cause of this reduction, because the loss of IGF-I mRNAs could be prevented by infusion of insulin from a miniosmotic pump after STZ treatment. IGF-I mRNA content per poly(A)⁺ RNA was significantly increased (*p* < 0.05) in insulin-treated vs vehicle-treated diabetic spinal cords. On the other hand, the poly(A)⁺ RNA content per RNA in spinal cord was unchanged whether rats were diabetic or treated with insulin. The glucose content was significantly reduced in the insulin-treated (8.9 ± 2.2 mmol/l, *n* = 4) vs vehicle-treated (44.9 ± 2.9 mmol/l, *n* = 4) diabetic rats.

Animal weights and glucose concentrations. IGF-I mRNA content is known to decline in starved animals [11, 12]. Because diabetic animals can weigh less, the effect of diabetes on animals weights and glucose concentrations, under the conditions of this study, was measured. Non-diabetic animals showed 4 % weight gain, but diabetic animals lost about 15 % of their initial weight in 2 weeks. There was a significant (*p* < 0.05) 20 % decrease in weights of diabetic (349 ± 8 g, *n* = 6 rats) relative to non-diabetic rats (438 ± 12 g, *n* = 6). Glucose concentrations were significantly (*p* < 0.05) elevated in diabetic (32.9 ± 2.1 mmol/l, *n* = 6) relative to non-diabetic (6.2 ± 0.3 mmol/l, *n* = 6) rats at 2 weeks post-treatment. In another study diabetic rats lost about 6 % (not significant) of their initial weight, and were 17 % lighter (*p* < 0.05, *n* = 5) than non-diabetic rats, after 8 days.

Discussion

IGF-I mRNA content was substantially and selectively diminished in liver, adrenals, and spinal cord of diabetic rats. Others have previously shown de-

creases in diabetic liver, kidney, heart, and lung [12, 13]. Taken together, these results support the suggestion that a reduction in IGF-I mRNA content is widespread among diabetic tissues. Certain syndromes of diabetes might be explained by this reduction.

IGF-I mRNA content in diabetic liver was selectively reduced. The reduction in circulating IGF-I concentration in diabetic rats [31–33] is likely to be closely tied to the profound fall in liver IGF-I mRNA. It is estimated that the 31 µg/day produced in liver could account for almost all of the circulating concentration of about 800 ng/ml [8, 34]. In the two 10-week diabetic rats, there appeared to be partial recovery of liver IGF-I mRNA content. This might have resulted from the slow regeneration of pancreatic islet cells, which is well known, but this possibility was not investigated.

IGF-I mRNA content was reduced in adrenal glands of only some rats at 1 and 2 weeks diabetes duration (not shown). However, at 5–10 weeks diabetes, IGF-I mRNA content was consistently and significantly reduced relative to total RNA, poly(A)⁺ RNA, and α-tubulin mRNAs in diabetic adrenal glands. The relative content of poly(A)⁺ RNA in total RNA from this tissue was unchanged.

The presence of IGF-I transcripts in human [35] and rat adrenal glands is consistent with the report that adrenal cells can secrete IGF-I [36]. Insulin and IGF receptors are present on chromaffin cells [36], suggesting that IGF-I may play an autocrine or paracrine role. The low IGF-I mRNA content in diabetic adrenal glands makes it difficult to avoid the implication of a potential relationship to the reduced epinephrine response to hypoglycaemia in diabetes [37, 38]. Involvement of chromaffin cells might be considered in the context of autonomic neuropathy. It is difficult to cleanly dissect medulla from cortex in the rat, and rat cDNA probes might be inappropriate to hybridize to adrenal RNA from other species because of the possibility of cross-hybridization at lower stringency to other members of the insulin/IGF gene family. Alternatively, *in situ* hybridization might be attempted. Further study is needed to establish whether the IGF-I mRNA is from cortex, medulla, or both tissues.

Autopsy reveals degeneration of the spinal cord in diabetic patients [39–41]. Unlike peripheral neuropathy, myelopathy is not presently considered a significant complication in diabetes. However, the case for involvement of the central nervous system is growing. For example, visual and auditory evoked potentials are abnormal early after onset of clinical diabetes [42]. Spinal cord degeneration [43], including reduction in perikaryal volume of ventral horn neurons [44], is also found in diabetic rats. Thus, rats may provide a useful experimental model in which to study myelopathy. The present observation of an early biochemical disturbance in IGF-I mRNA con-

tent in rat spinal cord adds to this growing list showing that the central nervous system is not spared in diabetes.

IGF-I mRNA content is significantly reduced per tissue, per total RNA, and per poly(A)⁺ RNA in spinal cord from diabetic rats. In a separate experiment (not shown), α -tubulin mRNA content was unchanged, providing further evidence for the selectivity of the decline in IGF-I mRNAs. These effects are observed after only 1 and 2 weeks of diabetes and rank among the earliest detectable biochemical changes observed in neural tissues.

It is striking that, under the same experimental conditions, conduction velocity is predicted and found to be reduced (at 1 and 2 weeks) in spinal cord of rats rendered diabetic with STZ [17]. A similar rate of decline in conduction velocity is observed in peripheral nerves. Loss of IGF-I mRNA in liver, as well as in spinal cord, may contribute to neuropathy. Infused IGFs can enhance nerve regeneration [45, 46], indicating that circulating IGF-I may support the nervous system. From this viewpoint, it may be pertinent that the kinetics of loss of IGF-I mRNA in liver is similar to the rate of decline of conduction velocity in spinal cord and peripheral nerves in a separate study with different animals [17]. Yang et al. [11] found somewhat slower kinetics in younger rats treated with higher STZ doses.

The prediction of Carsten et al. [17] was based on emerging evidence that insulin and IGFs may act together on neurons to support the neural circuitry (for greater detail see [15]). IGF-I mRNAs are present in brain [6, 7, 21] and spinal cord. Moreover, insulin [48, 49], IGF-I [50], and their receptors [51–53], are found in the CNS. These factors can support neurite outgrowth in cultured sensory, sympathetic [54], and motor neurons [55]. Survival of peripheral and central neurons is also supported [54, 56]. IGF-I increases sensory nerve regeneration distances [57] and motor axon sprouting [55] *in vivo*. Recently, a close correlation has been found between the development and regeneration of neuromuscular synapses and the expression of IGF-I [58] and IGF-II [59] genes.

Insulin and IGFs support neurite outgrowth in part by increasing expression of genes encoding structural proteins of axons and dendrites, such as tubulins [60] and neurofilaments [61]. Microtubules, comprised of tubulin subunits, are long, tubular elements of axons and dendrites contributing both to the cytoskeleton and to rapid anterograde and retrograde transport. The 68 and 170 kDa neurofilament proteins assemble into neurofilaments which form part of the axonal cytoskeleton, and are suggested to regulate the diameter of axons [62].

These observations may be significant in understanding the pathogenesis of this enigmatic disorder [63, 64]. There can be loss of ganglion cells, de-

creased numbers and diameters of axons, diminished transport [65], and reduced conduction velocity in diabetic neuropathy. These pathological changes might be considered in light of the decline in serum IGF-I, evident when the data are corrected for age in diabetic patients [66]. Rhesus monkeys provide an interesting model of non-insulin-dependent diabetes [67]. Young, lean monkeys begin with normal glucose and insulin levels. As animals age, weight is gained, insulin levels become elevated, and impaired glucose tolerance begins to emerge, which progresses to overt hyperglycaemia. It is striking that serum IGF-I levels are progressively reduced in parallel with these age-dependent changes, but are not correlated with either glycaemic state or insulinaemia. Neuropathy is seen in diabetic rhesus monkeys [68]. A more detailed account of the theory linking IGF activity to the pathogenesis of experimental and clinical diabetic neuropathy will appear elsewhere [16]. Acute episodes in insulin-dependent diabetes are associated both with reduced IGF-I activity and transient neuropathy [69, 70]. At the same time, although these may be contributory factors, it is prudent to consider that diabetes is a complex disorder, and neuropathy is believed by many to arise as a direct or indirect result of hyperglycaemia. Further study of this provocative correlation between IGF activity and neuropathy is needed.

IGF-I protein and mRNA contents in liver probably do not decline as a result of STZ toxicity. For example, the administration of insulin following STZ induction of diabetes can antagonize the decline in both IGF-I mRNA and circulating somatomedin [31, 71]. Moreover, insulin can restore serum IGF-I levels towards normal in pancreatectomized dogs [72], showing that this capacity of insulin to correct IGF-I concentrations is present in diabetic tissues never exposed to STZ.

Moreover, the IGF-I mRNA content in spinal cord probably did not decline as a result of STZ toxicity. However, one may not entirely exclude the possibility that insulin can increase IGF-I mRNA content in diabetic spinal cord directly, rather than by preventing STZ toxicity. Insulin ameliorated the loss of IGF-I mRNAs that occurred in STZ-treated diabetic rats. Insulin also prevents impairment of conduction velocity in STZ-treated diabetic rats [17].

The reduced IGF-I mRNA content in liver is due in part to a decline in growth hormone secretion in rats [73]. There is tissue resistance to growth hormone as well, because treatment of diabetic rats with growth hormone does not induce IGF-I [32]. Tissue resistance is not due to a reduction in growth hormone receptor mRNA in liver [12], and growth hormone binding remains normal in rats diabetic for 1 week, although IGF-I levels are reduced [31]. Much less is known about the basis for the decline in IGF-I mRNA content in diabetic tissues other than liver.

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