Insulin-Like Growth Factor (IGF) Gene Expression Is Reduced in Neural Tissues and Liver from Rats with Non-Insulin-Dependent Diabetes Mellitus, and IGF Treatment Ameliorates Diabetic Neuropathy

HUI-XIN ZHUANG, LAURA WUARIN, ZHI-JIAN FEI and DOUGLAS N. ISHII

Department of Physiology and Department of Biochemistry and Molecular Biology, Colorado State University, Fort Collins, Colorado

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ABSTRACT

Neural disturbances are observed in the peripheral and central nervous systems of patients with insulin-dependent diabetes mellitus (IDDM) and non-IDDM (NIDDM). Insulin-like growth factors (IGFs) are neurotrophic growth factors that can support nerve regeneration and neuronal survival in the types of neurons known to be afflicted in diabetes. We tested the hypotheses that IGF gene expression is reduced in neural tissues and liver of spontaneously diabetic obese Zucker (fa/fa) rats and that IGF treatment can prevent neuropathy. There was a significant early reduction in IGF-II mRNA content as measured per mg of wet tissue or per poly(A) RNA in sciatic nerves, spinal cord and brain from spontaneously diabetic obese (fa/fa) vs. nondiabetic lean (+/+) adult rats. In addition, IGF-I mRNA content was reduced in liver but not nerve or spinal cord of NIDDM rats. Pain/pressure thresholds were abnormal (hyperalgesia) in diabetic (fa/fa) vs. nondiabetic (+/+) rats, and subcutaneous infusion of IGF-II restored thresholds toward normal. The low dose of IGF-II that prevented hyperalgesia in contrast to its effect in diabetic rats was ineffective in nondiabetic rats. IGF-II gene content was reduced in liver but not nerve or spinal cord of diabetic (fa/fa) rats and subcutaneous infusion of IGF-II restored thresholds toward normal.

Diabetic neuropathy is a complication that can afflict the sensory, autonomic and motor nervous systems in both IDDM and NIDDM (Dyck et al., 1987; Thomas and Tomlinson, 1993; Vinik et al., 1992). The most common form of this complication is a sensory polyneuropathy with symptoms such as paresthesia, unrelenting pain (hyperalgesia) and reduced temperature and vibration perception thresholds. There is a progressive deterioration with age resulting in loss of synapses, axons and neurons. Gastroparesis, bladder atony, cardiovascular dysfunction and impotence are some consequences of autonomic neuropathy. Muscle atrophy and weakness are observed in motoneuropathy.

The central nervous system is not spared, and depression, phobias, anorexia (Lustman et al., 1988; Popkin et al., 1988), loss of memory and reduction in complex reasoning skills (Perlmuter et al., 1984; Ryan, 1988) are more prevalent in the diabetic than the general population. Progressive brain atrophy (Araki et al., 1994), loss of axons and degeneration of cortical neurons is observed (Jakobsen et al., 1987; Reske-Nielsen et al., 1965; Soininen et al., 1992). Autopsy reveals loss of motor and sensory neuron perikarya and degeneration of long tracts in the spinal cord (Reske-Nielsen et al., 1965; Slager and Webb, 1973; Woltman and Wilder, 1929). It is widely believed that these diabetic neural disturbances may be a secondary consequence of hyperglycemia, but this remains a controversial issue (Greene, 1987; Strowig and Raskin, 1992).

IGFs are neurotrophic factors capable of supporting neurite outgrowth and survival in a wide variety of peripheral and central neurons (Ishii, 1993; Recio-Pinto and Ishii, 1988a). IGF-II gene expression is higher in brain, spinal cord (Brown et al., 1986; Soares et al., 1986) and nerve (Glazner et al., 1994) than in other tissues of the adult rat. Overexpression of the IGF-I gene in transgenic mice results in a disproportionate increase in brain size (Mathews et al., 1988). IGF increases but an anti-IGF antibody decreases sciatic nerve regeneration (Glazner et al., 1993; Near et al., 1992). Moreover, IGF enhances but the anti-IGF antibody reduces the survival of motoneurons after axotomy in rats (Pu et al., 1995). Occupancy of IGF receptors (Recio-Pinto and Ishii, 1988b) is closely correlated with the capacity of IGFs to increase neurite outgrowth as well as the expression of genes encoding axonal cytoskeletal proteins such as tubulins and neurofilaments (Mill et al., 1985; Wang et al., 1992).

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ABBREVIATIONS: IGF, insulin-like growth factor; IDDM, insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus;
A recent theory proposes that an age- and disease-dependent decline in redundant IGF neurotrophic support may be pathogenic for progressive diabetic neural disturbances (Ishii, 1995). Circulating IGF-I levels are reduced in rats with streptozotocin-induced diabetes (Baxter et al., 1979; Phillips and Young, 1976). In this model of IDDMD, IGF mRNA content is reduced in nerves (Wuarin et al., 1994), liver and spinal cord (Ishii et al., 1994). Subcutaneous infusion of IGF-I or IGF-II prevents neuropathy in IDDMD rats independently of hyperglycemia (Ishii and Lupien, 1995; Zhuang et al., 1996).

The nature and extent of biochemical pathology within the diabetic nervous system are not fully known, particularly in NIDDM, which afflicts 90% of diabetic patients. Weight loss and hypoinsulinemia are associated with IDDMD, whereas weight gain and hyperinsulinemia are associated with NIDDM. Is IGF gene expression reduced in neural tissues in NIDDM as well as in IDDMD? The following interrelated hypotheses were tested in the obese, hyperinsulinemic and spontaneously diabetic Zucker (fa/fa) rat model of NIDDM: (1) IGF gene expression is reduced in neural tissues in NIDDM, (2) IGF gene expression is reduced in liver, the main source of circulating IGF and (3) IGF replacement therapy can prevent hyperalgesia independently of hyperglycemia in NIDDM.

**Experimental Procedures**

**Materials.** Glucose Diagnostic Kit 510A was obtained from Sigma Chemical (St. Louis, MO). Recombinant human IGF-II obtained from GroPep (Adelaide, Australia) was >95% pure by high performance liquid chromatography. Miniosmotic pumps were purchased from ALZA (Palo Alto, CA).

**Animal procedures.** Inbred male Zucker rats lean, nonobese (ZDF Gmi+/+ or +/+ ) and obese, diabetic (ZDF Gmi fa/fa) were purchased from Genetic Models (Indianapolis, IN). The diabetic rats, which had been inbred for >25 generations from a group of obese (fa/fa) rats that were spontaneously diabetic, develop hyperglycemia at ~7 weeks of age and display elevated glycated hemoglobin (HbA1) levels. They become hyperinsulinemic at 10 to 13 weeks of age (Peterson et al., 1990). Purina 5008 rat chow and water were provided ad libitum. The fa/fa rats were randomly assorted into treatment groups at 11 weeks of age. Plasma glucose concentrations were determined (see figure legends).

Rats were implanted (subcutaneously in midback) with a miniosmotic pump (0.5 μl/hr) that contained vehicle (1 mM acetic acid, pH 6) or IGF-II (400 μg/ml) for 2 weeks. All solutions were sterilized by passage through 0.2-μm filters.

**Paw pressure test.** A paw pressure test was conducted with an Analgesy-Meter (Ugo Basile, Varese, Italy). It has been used to measure hyperalgesia in the streptozotocin diabetic rat model of chronic pain (Courteix et al., 1993; Wuarin-Bierman et al., 1985), in which it seems likely that both pain- and pressure-responsive fibers are activated. A force that increases at a constant rate was applied to the center of a hind paw. A nociceptive response causes the rat to withdraw its paw, and the pain/pressure tolerance threshold was read from a linear scale. A value consisted of the mean of four repeat measurements. Rats were handled daily before initiation of the experiments to minimize any impact of handling-related stress.

**RNA preparation, electrophoresis and transfer to nitrocellulose.** The entire brain (except olfactory bulb), liver and spinal cord were excised. The sciatic nerve was excised from a point ~10 mm above the sciatic notch to the entry of the major branches of the sural, tibial and common peroneal nerves into their respective muscles. Ganglia were not included. Total RNA was immediately extracted from various tissues according to the method of Chomczynski and Sacchi (1987); LiCl was used to remove glycogen from liver samples (Puissant and Houdéne, 1990). RNA concentration and purity were estimated by A260nm and A280nm. RNA yield was linear with tissue weight, permitting calculation of total RNA content per tissue.

Equivalent amounts of RNA (30 μg) were electrophoresed through 0.8% agarose gels containing formaldehyde as previously described (Lehrach et al., 1977). 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 (Thomas, 1980). Alternatively, samples were incubated for 15 min at 60°C in 14% formaldehyde and 7.5× standard saline citrate (1× standard saline citrate ~ 150 mM NaCl, 15 mM sodium citrate, pH 7), rapidly cooled on ice and directly loaded (8 μg in duplicate) onto nitrocellulose using a slot-blot filtration device (Schleicher & Schuell, Keene, NH) in a manner similar to that described for dot blots (Kafatos et al., 1979). Nitrocellulose blots were baked at 80°C for 2 hr under vacuum and cross-linked to RNA using UV light at 0.12 J/cm² (Stratagene, La Jolla, CA). Various concentrations (0, 2, 4, 6, 8 and 10 μg) of an RNA standard were included on each slot-blot to determine the linear range of autoradiographic exposure. RNA retention was linear to ~10 μg/slot.

**cDNA clones.** An IGF-I cDNA clone in pUC9 (gift of Dr. Argiris Efstratiadis) contains the rat pre-pro-IGF-I coding sequence. This clone begins with nucleotide 1780 of exon 1, contains no sequences from exon 4 and ends in nucleotide 718 of exon 5 (Shimatsu and Rotwein, 1987). The rat IGF-II cDNA clone 27 in pUC9 contains the entire rat pre-pro-IGF-II coding sequence plus 61 nucleotides of 5′ and 3′ nucleotides of 3′ sequence (Soares et al., 1985). Plasmid DNA was isolated from minipreps (Wang et al., 1992) and purified on NACS.37 columns.

**Hybridization to RNA.** Oligonucleotide 20-mer primers were synthesized flanking the 5′ and 3′ multiple cloning sites of pUC9 plasmids containing cDNAs of interest (Runnebaum et al., 1991). Polymerase chain reaction (Saiki et al., 1985) was used to amplify cDNA inserts, which were electrophoresed in low-melting-temperature agarose gels. The desired bands were excised, extracted with phenol-chloroform and ethanol-precipitated. The purified inserts and one of the polymerase chain reaction primers were then used in primer extension with Taq polymerase (40 cycles) to produce 32P-labeled (1–3 × 10⁹ dpm/μg) single-stranded antisense cDNA probes (Mizobuchi and Frohman, 1992). The specificity of IGF-I and IGF-II cDNA probes has been established (Glazner et al., 1994; Wuarin et al., 1994). Nitrocellulose blots were hybridized to 32P-labeled cDNA probes for 16 to 18 hr at 42°C and then washed using a procedure to detect low-abundance transcripts (Sambrook et al., 1989). Blots were stripped to remove all hybridized cDNA, as indicated by autoradiography, and rehybridized to end-labeled oligo(dT)-18 probes (Harley, 1987) to measure poly(A)⁺ RNA. The hybridization of oligo(dT) on slot-blots was linear with RNA concentrations between 2 and 10 μg.

**Analysis and quantification of RNA blots.** Autoradiograms were prepared on X-ray film using Cronex Lightning Plus enhancing screens at ~70°C. The densitometric images were captured and analyzed using MicroScan 1000 gel analysis software (Technology Resources, Nashville, TN). The linear range for measurements was determined by including a concentration series of RNA on blots and taking several autoradiographic exposures of different duration. Results are reported in relative densitometric units (arbitrary) in figures. IGF mRNA/total RNA was calculated by dividing the IGF mRNA relative densitometric values by total RNA loaded/sample; poly(A)⁺ RNA/total RNA was calculated by dividing the oligo(dT) values by total RNA loaded/sample; IGF mRNA/poly(A)⁺ RNA was calculated by dividing the former by the latter; and IGF/mg was calculated by multiplying the IGF mRNA/total RNA by the total RNA/mg of wet weight tissue. These methods permit calculation of the change in IGF mRNA content relative to the pool of total mRNA.
total RNA and wet weight tissue. One can also examine for changes in total mRNA pool size relative to total RNA or tissue wet weight.

**Statistical analysis.** All data were subjected to the Newman-Keuls post hoc test for the presence of interactions between groups with the use of CSS:Statistica (StatSoft, Tulsa, OK). Values for P show significant differences between group means and are mean ± S.E.

**Results**

**Profile of IGF mRNAs in liver and brain from diabetic and nondiabetic Zucker and Sprague-Dawley rats.** Taq primer extension was used to prepare single-stranded antisense IGF-I and IGF-II cDNA hybridization probes. Their specificity has been studied in various tissues with the use of Northern and slot-blot hybridizations (Glazner et al., 1994; Glazner and Ishii, 1995; Wuarin et al., 1994). The rat IGF-I and IGF-II genes are each unique but give rise to transcripts of varying sizes due to multiple promoters and alternative polyadenylation sites. IGF-I mRNAs of 0.7 to 1.1, 1.8, 2.6, 3.9 and 7.0 kb and IGF-II mRNAs of 1.8, 2.2, 4.8, 5.0, 5.3 and 6 kb can be produced in varying amounts in different tissues. IGF gene structure has been previously reviewed (Sussembach et al., 1991).

Northern blots containing RNA from liver of adult Zucker (fa/fa and +/+ ) rats showed the same IGF-I mRNA bands as previously observed in Sprague-Dawley rats (fig. 1, top). IGF-I mRNA levels were lower in liver from diabetic (fa/fa) vs. nondiabetic (+/+ ) as well as from streptozotocin-induced diabetic Sprague-Dawley vs. nondiabetic Sprague-Dawley rats, but quantification was needed. IGF-I mRNA is much less abundant in adult brain than liver and can be detected only with prolonged autoradiographic exposures.

IGF-II gene expression is developmentally down-regulated and in very low abundance in adult liver. The same IGF-II mRNA bands were detected in RNA from brains obtained from the two rat strains, except that a minor band was more pronounced in Zucker relative to Sprague-Dawley rats and increased in diabetic (fa/fa) relative to nondiabetic (+/+ ) (fig. 1, bottom). Thus, the appropriate tissue-selective profiles of IGF transcripts were observed, and nonspecific cross-hybridization was not detected.

**IGF mRNA content in sciatic nerve.** Equivalent amounts of total RNA from the sciatic nerves of 13-week-old spontaneously diabetic (fa/fa) and nondiabetic (+/+ ) rats were loaded onto slot-blot hybridizations for quantification of gene expression. The blots were hybridized to detect IGF-II transcripts (fig. 2). IGF-II mRNA/mg of wet weight was significantly reduced (P < .005) in diabetic (fa/fa) vs. nondiabetic (+/+ ) nerves. The blots were stripped and rebybridized to oligo(dT) to measure the pool of total poly(A)+ RNA transcripts. IGF-II mRNA/oligo(dT) was significantly reduced (P < .02) in (fa/fa) vs. (+/+ ) nerves. The oligo(dT) content/mg of nerve was unchanged.

The same nerve RNA samples were hybridized to detect IGF-I transcripts (fig. 3). A significant reduction in IGF-I mRNAs was not detected regardless of whether measured per mg of tissue or per oligo(dT).

**IGF mRNA content in spinal cord.** IGF gene expression was further studied in tissues from the central nervous system. Both IGF-II mRNA content/mg of tissue and IGF-II mRNA content/oligo(dT) were significantly decreased (P < .02) in RNA from spinal cord of NIDDM (fa/fa) vs. nondiabetic (+/+ ) rats (fig. 4). IGF-I mRNA content was reduced ~30%, but this reduction did not reach significance (fig. 5). The oligo(dT) content/mg of spinal cord was unchanged.

**IGF-II mRNA content in brain.** With respect to brain, IGF-II mRNA content/mg of tissue and IGF-II mRNA content/oligo(dT) were significantly reduced (P < .01) in diabetic (fa/fa) vs. nondiabetic (+/+ ) rats (fig. 6). The oligo(dT) content/mg of tissue was unchanged. IGF-II mRNA was examined because IGF-II is the predominant IGF in brain. IGF-I gene expression has been studied by others (Bornfeldt et al., 1989) and was not reexamined.

**IGF-I mRNA content in liver.** IGF-I gene expression was measured in liver because liver is the main source for circulating IGPs. In contrast, IGF-II gene expression is developmentally down-regulated and very low or undetectable in adult rat liver (Brown et al., 1986; Soares et al., 1985).

There was a significant reduction in IGF-I mRNA content/mg of wet weight tissue (P < .02) as well as IGF-II mRNA content/oligo(dT) (P < .01) in RNA from livers of diabetic (fa/fa) vs. nondiabetic (+/+ ) rats (fig. 7). The oligo(dT) content/mg of tissue was unchanged.

**NIDDM rats developed hyperalgesia, and hyperalgesia is overcome by systemic IGF-II administration.** A paw pressure test was conducted with an Analgesy-Meter, which applies a force that increases at a constant rate to the center of a hind paw. Hyperalgesia is shown as a reduction in the force causing paw withdrawal in streptozotocin-induced rats.
The pain/pressure threshold was determined in age-matched rats randomly assigned to treatment groups. Diabetic rats were implanted at 11 weeks of age with subcutaneous miniosmotic pumps that released either vehicle or IGF-II for 2 weeks. The paw-pressure thresholds were significantly decreased \( (P < .01) \) in vehicle-treated diabetic \((fa/fa)\) vs. nondiabetic \((+/+)\) 13-week-old rats (fig. 8). Hyperalgesia was ameliorated (paw-pressure thresholds returned toward normal) in IGF-II treated vs. vehicle-treated diabetic rats \((P < .02)\).

**Fig. 2.** Sciatic nerve IGF-II mRNA content in NIDDM rats. Sciatic nerves from 13-week-old spontaneously diabetic \((fa/fa)\) and nondiabetic lean \((+/+\) or \(+/fa)\) Zucker rats were excised and weighed. Equivalent amounts of RNA \((8 \mu g/slot\) in duplicate) were loaded onto slot-bLOTS and hybridized to detect IGF-II mRNAs. Blots were stripped and rehybridized to oligo(dT) to measure poly(A)\(^{+}\) RNA. Autoradiograms were analyzed with a densitometer connected to a computer, and the densitometric volume was quantified in relative arbitrary units. The IGF-II mRNA content/mg of wet weight tissue or IGF-II mRNA content/oligo(dT) was normalized relative to nondiabetic values. Values are mean \( \pm \) S.E. \((n = 4\) samples, each containing four pooled nerves from two rats). \( *P < .02. \) \( **P < .005. \)

IDDM rats (Courteix et al., 1993; Wuarin-Bierman et al., 1987).

The pain/pressure threshold was determined in age-matched rats randomly assigned to treatment groups. Diabetic rats were implanted at 11 weeks of age with subcutaneous miniosmotic pumps that released either vehicle or IGF-II for 2 weeks. The paw-pressure thresholds were significantly decreased \((P < .01)\) in vehicle-treated diabetic \((fa/fa)\) vs. nondiabetic \((+/+)\) 13-week-old rats (fig. 8). Hyperalgesia was ameliorated (paw-pressure thresholds returned toward normal) in IGF-II treated vs. vehicle-treated diabetic rats \((P < .02)\).
Low doses of IGF-II had no effect on hyperglycemia or obesity in NIDDM rats. Further tests were conducted on the animals described in figure 8. Hyperglycemia was present \((P < .001)\) in diabetic \((fa/fa)\) vs. nondiabetic \((+/- or +/fa)\) rats (table 1). However, the serum glucose concentrations remained essentially the same in IGF-II vs. vehicle-treated diabetic rats. The diabetic rats were significantly heavier than the nondiabetic rats \((P < .01)\). IGF-II treatment did not reduce obesity in diabetic rats (table 1).

Discussion
Comparison of IGF gene expression in brain, spinal cord and nerves of NIDDM and IDDM rats. These results show that IGF-II gene expression was significantly reduced in nerve (fig. 2), spinal cord (fig. 4) and brain (fig. 6) of spontaneously diabetic \((fa/fa)\) relative to nondiabetic \((+/- or +/fa)\) rats. These changes were selective because neither poly(A)\(^{+}\) RNA nor IGF-I mRNA content was reduced in nerve or spinal cord. The alterations in IGF-II gene expression were associ-
ated with the presence of sensory neuropathy measured as an abnormal paw pressure threshold (hyperlgesia). The pattern of IGF gene expression can be compared in NIDDM and IDDM rats. IGF-II gene expression is significantly reduced in nerve (Wuarin et al., 1994), spinal cord and brain (Wuarin et al., 1996) in IDDM as well as in NIDDM. The IDDM rat differs from NIDDM rats, however, in that IGF-I mRNA content is additionally reduced in nerve (Wuarin et al., 1994) and spinal cord (Ishii et al., 1994). This difference might be due to the hypoinsulinemia in IDDM not shared with NIDDM. The insulin resistance in NIDDM may be insufficient to reduce IGF-I mRNA content significantly, albeit a trend was observed in spinal cord (fig. 5). IGF-I mRNA content in brain is reported to be unchanged in IDDM rats (Bornfeldt et al., 1989).

In situ hybridization was used to localize IGF-I and IGF-II mRNAs mainly to Schwann cells but also to a few macrophages in nerves (Pu et al., 1995). It has been used to detect the high abundance of IGF-II mRNAs in leptomeninges, choroid plexus and brain microvasculature (Ichimiya et al., 1988; Hynes et al., 1988; Stylianosolou et al., 1988). We currently

![Fig. 6. Brain IGF-II mRNA content in NIDDM rats. Brain RNA from NIDDM (fa/fa) and nondiabetic (+/+) or +/+fa) rats were hybridized to detect IGF-II mRNAs. Blots were stripped and rehybridized to oligo(dT). Values are mean ± S.E. (n = 8 rats). **P < .01. ***P < .0001.](image)

![Fig. 7. Liver IGF-I mRNA content in NIDDM rats. Liver RNA from NIDDM (fa/fa) and nondiabetic (+/+) or +/+fa) rats were hybridized to detect IGF-I mRNAs. Blots were stripped and rehybridized to oligo(dT). Values are mean ± S.E. (n = 8 NIDDM, 7 nondiabetic rats). *P < .02. **P < .01.](image)
interpret our slot-blot results as showing that IGF-II gene expression is likely to be reduced in these brain regions but do not exclude the possibility that IGF-II mRNAs may be reduced in other regions as well. The previously held beliefs that IGF-II mRNA is produced only in leptomeninges, choroid plexus and brain microvessels and that the low-level expression detected elsewhere in brain parenchyma is due to contamination from leptomeninges are no longer tenable. This conclusion is based on the observation that the paternal imprinting of the IGF-II gene in peripheral tissues does not extend to brain (DeChiara et al., 1991). For example, the IGF-II mRNA detected by allele-specific methods in cerebellum and cortex arises from the maternal allele, and this result could not be due to contamination from leptomeninges, in which only the paternal allele is expressed (Hu et al., 1995). IGF-II mRNA is found in glia (Rotwein et al., 1988) and neurons in hippocampus, thalamus and cortex (Logan et al., 1994).

The mechanism for decline in IGF-II mRNA content in diabetic neural tissues is not known; it might be related to the reduction in growth hormone in IDDM rats (Harrison and Robinson, 1980); intraventricular administration of growth hormone can partially restore IGF-II mRNA levels in hypophysectomized rats (Hynes et al., 1987). Insulin treatment incompletely restores IGF-I and IGF-II mRNA content in nerve (Wuarin et al., 1994) and IGF-I mRNA in spinal cord (Ishii et al., 1994) in IDDM rats.

Circulating IGFs in NIDDM and IDDM rats. IGF-I mRNA content is reduced in livers of both IDDM (Bornfeldt et al., 1989; Fagin et al., 1989) and NIDDM (fig. 7) rats. It is estimated that the 31 μg/day produced in liver may account for all of the circulating IGF-I (Schwander et al., 1983; Scott et al., 1985), and circulating IGF-I levels are reduced in IDDM rats (Phillips and Young, 1976; Baxter et al., 1979). Insulin treatment partially restores hepatic IGF-I mRNA content and circulating IGF-I levels (Boni-Schnetzer et al., 1989; Fagin et al., 1989). The level of circulating IGF-II is normally rather low in adult rats, probably due to developmental down-regulation of IGF-II gene expression in liver (Brown et al., 1986; Soares et al., 1985).

IGFs are sufficiently small (molecular mass ~ 7–7.5 kDa) that circulating free IGFs can access sensory and sympathetic ganglia through fenestrated capillaries (Jacobs, 1977; Jacobs et al., 1976). They can also act on motor nerve terminals (Caroni and Grandes, 1990).

Overall, there is a profound early loss of autocrine/paracrine and endocrine sources of IGFs that precedes damage to the nervous system in IDDM (Ishii et al., 1994; Wuarin et al., 1994) and probably NIDDM rats. The decrease in IGF gene expression is independent of weight gain (NIDDM) or loss (IDDM) and of hyperinsulinemia (IDDM) or hypoinsulinemia (NIDDM).

IGF treatment protects against diabetic neuropathy. The NIDDM rats had abnormal paw/pressure thresholds (fig. 8). Subcutaneous infusion of IGF-II restored thresholds toward normal. Treatment with either IGF-I or IGF-II was found to arrest the progression of hyperalgesia in IDDM rats as well (Zhuang et al., 1996).

Both myelinated and unmyelinated axons undergo degeneration and loss (Archer et al., 1983; Said et al., 1983), and axonopathy is the predominant neuropathological lesion in diabetes. Nerve regeneration is impaired in IDDM rodents (Ekstrom and Tomlinson, 1989; Longo et al., 1986), and this can be reversed by subcutaneous infusion of IGFs (Ishii and Lupien, 1995; Zhuang et al., 1996). These studies taken together show that IGF treatment may protect against neuropathy in NIDDM as well as IDDM.

IGF-II acts selectively on IGF rather than insulin receptors at low doses. After nerve crush, the local application of small doses of IGF-II but not insulin can increase nerve regeneration in nondiabetic rats (Glazner et al., 1993; Near et al., 1992). Physiological doses of IGFs that induce neurite outgrowth bind to IGF receptors but do not cross-occupy insulin receptors on human neuroblastoma cells (Recio-Pinto and Ishii, 1988b).

Low doses of IGFs protect independently of hyperglycemia in NIDDM and IDDM rats. Treatment with low doses (4.8 μg/day/rat) of IGF-II protected against neuropathy independently of continued hyperglycemia in NIDDM rats (table 1). It also had no effect on obesity. Similar low doses of

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**TABLE 1**

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<tr>
<th>Treatment</th>
<th>Serum glucose (mmol/liter)</th>
<th>Body weight (g)</th>
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<tbody>
<tr>
<td>Nondiabetic</td>
<td>3.3 ± 0.5</td>
<td>314.6 ± 3.6</td>
</tr>
<tr>
<td>Diabetic + vehicle</td>
<td>29.4 ± 1.5*</td>
<td>391.9 ± 10.4*</td>
</tr>
<tr>
<td>Diabetic + IGF-II</td>
<td>26.6 ± 1.5*</td>
<td>372.3 ± 13.2*</td>
</tr>
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NIDDM and nondiabetic rats were treated as described in figure 8. The serum glucose concentrations and body weights were measured after 2 weeks of treatment. Values are mean ± S.E. (n = 8 rats per group).

*IGF-II or vehicle-treated NIDDM (fa/fa) group mean significantly different from that of nondiabetic (+/+ or +/+fa) rats (P < 0.001).

**IGF-II or vehicle-treated NIDDM (fa/fa) group mean significantly different from that of nondiabetic (+/+fa) rats (P < 0.01).**
IGF-I and IGF-II prevented neuropathy in IDDM rats independently of hyperglycemia or weight loss (Ishii and Lupien, 1995; Zhuang et al., 1996).

On the other hand, supraphysiological doses (>300 μg/day/rat) of IGF-I can prevent weight loss in IDDM rats (Carlsson-Skwirut et al., 1989; Scheiwiller et al., 1986). Hyperglycemia was observed to persist in these studies as well.

**Implications for clinical diabetic neuropathy.** The peripheral neuropathy differs between IDDM and NIDDM. Fiber atrophy is more severe and diffuse in clinical and experimental IDDM than in NIDDM (Sima et al., 1988; Yagi-hashi et al., 1995). This may be related to the observation that nerve IGF-I mRNA content is reduced in IDDM (Wuarin et al., 1994) but not in NIDDM (fig. 3). In addition, IGF-I mRNA content appears to be higher than IGF-II mRNA content in nerves, although this is based on an assumption of equal efficiency of probe hybridization and is difficult to precisely assess. If this were correct, it would suggest that a decline in IGF-I mRNA content may be of greater consequence to nerve regeneration than a decline in IGF-II mRNA. Indeed, nerve regeneration is impaired in IDDM (Ekstrom and Tomlinson, 1989; Ishii and Lupien, 1995) but not in the Zucker (fa/fa) NIDDM model.

In nondiabetic rats, the elongation of regenerating axons in nerves is correlated primarily with an upregulation of IGF-I mRNAs at and distal to a site of nerve lesion (Glazner et al., 1994; Pu et al., 1995). By contrast, IGF-II mRNAs are upregulated mostly near the terminals of nerves and in muscle where they are believed to play a role in nerve terminal sprouting preceding polynuclear innervation during the establishment of neuromuscular synapses (Glazner and Ishii, 1995; Pu et al., 1995). The observation that subcutaneous infusion of IGF-II as well as IGF-I can increase impaired nerve regeneration in diabetic rats may be a pharmacologic consequence of the fact that both IGFs can activate the type I IGF receptor in nerve. However, the IGF receptor mediating these effects cannot be identified from the present results.

Circulating IGF-I levels are reduced in half in IDDM and NIDDM patients (Tan and Baxter, 1986). Superimposed on this reduction is a slow, continuous decline in IGF-I levels over decades (Hall and Sara, 1984; Tan and Baxter, 1986), which is proposed to be linked to the age-dependent emergence of diabetic neuropathy (Ishii, 1995). Moreover, circulating IGF-I levels are reduced to a greater extent in diabetic patients with vs. without neuropathy (Migdalas et al., 1995). A similar continuous decline in circulating IGF-I levels is seen in rhesus monkeys as they advance from young, lean and nondiabetic to old, obese and diabetic animals (Bodkin et al., 1991); neuropathy is observed (Cornblath et al., 1989).

The finding that IGF administration can prevent neuropathy in NIDDM and IDDM rats may be good news, particularly for the many diabetic patients unable to adequately regulate their hyperglycemia. Even with intensive insulin therapy, a large fraction of IDDM patients continue to deteriorate in neural function, and adjunct IGF treatment may be useful. It is cautioned that only careful clinical trials can establish the safety and efficacy of IGF medication for the prevention of diabetic neuropathy. Patients should continue to strive to maintain normal glycemia to reduce the risk of various diabetic complications.

**References**


