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Neurotrophins and Peripheral Neuropathy

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Neurotrophins and peripheral neuropathy

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SUMMARY

The most common form of peripheral neuropathy is that associated with diabetes mellitus. In rodent models of diabetes there are expression deficits in nerve growth factor (NGF) and in its high-affinity receptor, trkA, leading to decreased retrograde axonal transport of NGF and decreased support of NGF-dependent sensory neurons, with reduced expression of their neuropeptides, substance P and calcitonin gene-related peptide (CGRP). Treatment of diabetic rats with intensive insulin normalized these deficits and treatment with exogenous NGF caused dose-related increases, giving levels of NGF and neuropeptides which were greater than those of controls. Neurotrophin-3 (NT-3) mRNA was also deficient in leg muscle from diabetic rats and administration of recombinant NT-3 to diabetic rats increased the conduction velocity of sensory nerves without affecting motor conduction velocity. These findings implicate deficient neurotrophic support in diabetic neuropathy and suggest that its correction should be a paramount therapeutic target.

1. INTRODUCTION

Distal symmetrical polyneuropathy is the commonest presentation of diabetic neuropathy. Its symmetrical nature is consistent with pathogenesis in the form of disseminated biochemical anomalies to which neurons are especially vulnerable. The polyneuropathy indicates that more than one type of peripheral nerve can be affected, though sensory defects predominate in the symptoms. The distal origin indicates that problems begin to be manifest at the extremities. One other clinical feature is informative; this neuropathy is described as showing a 'stocking-glove' distribution, with the feet and hands presenting the earliest and most severe signs and symptoms. This indicates that neurons with the longest axons are the most vulnerable. Taken together, these clinical features point to an aetiology in which some biochemical defect attributable to diabetes mellitus provokes secondary neurochemical defects, which are relatively non-selective towards different classes of peripheral nerves, but which afflict those with the requirement to maintain long peripheral (and central) projections.

Virtually all of the biosynthetic capacity of the neuron is retained close to the source of messenger RNA (mRNA) in the nucleus and this requires the translocation of macromolecules and organelles from their site of synthesis to the axonal extremity. In some primary afferents and motoneurons of the lower limb in a tall individual, this represents a cytological marathon of over 1 m. Maintenance of this process of intra-axonal transport is an absolute requirement for the survival of the distal axon and the degeneration consequent on experimental interruption of transport resembles the degeneration seen in several peripheral neuropathies (Cuénod *et al.* 1972). Thus it is not surprising that possible deficits in anterograde axonal transport have been explored as potential components of the aetiology of diabetic neuropathy, given the vulnerability of the longest axons as described above.

In brief, this work has revealed that many components of axoplasm are delivered to the periphery in reduced amounts in rats with experimental diabetes (for a review, see Tomlinson & Mayer 1984). However, it appears that the process of translocation itself functions more or less normally in experimental diabetes (Robinson *et al.* 1987; Tomlinson *et al.* 1988), so that attention is now more properly focused on deficits in synthesis of material in the nerve cell body: effective transport with deficient production is equally detrimental to export. In neurons regulation of the synthesis of the various classes of protein is adjusted via production of neurotrophic factors by the neuronal target cells and their delivery to the cell body by retrograde axonal transport. In this way the nature and activity of the target cell maintains appropriate expression of the phenotype of its innervating neurons.

Nerve growth factor (NGF) is a member of a family of neurotrophic factors that includes brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3) and neurotrophin 4/5 (NT-4/5) (for a review, see Ebendal 1992). NGF is retrogradely transported in sensory and sympathetic neurons in adult rats (Hendry *et al.* 1974; Schmidt & Yip 1985) and about 50% of the adult rat lumbar sensory neurons can bind NGF with high affinity (Richardson *et al.* 1986). BDNF and NT-3 are also retrogradely transported from an injection site in the sciatic nerve to the dorsal root ganglia (DRG) and motoneurons of adult rats (DiStefano *et al.* 1992).

There is mounting evidence to suggest that NGF and the other neurotrophins are involved in maintaining and fine tuning expression of the mature phenotype in adult neurons. Deprivation of trophic support by

axotomy provokes a pattern of change that has been defined as somatofugal atrophy (Hoffman *et al.* 1987). Many of the changes in the cell body of an axotomized nerve can be attenuated or prevented by administration of exogenous neurotrophin, implicating trophic deficits causally, though at the time of writing this approach has been explored extensively only with NGF. Sciatic nerve transection results in a decrease in mRNA for the medium neurofilament protein (NF-M) in large DRG neurons as measured by *in situ* hybridization (Verge *et al.* 1990). Administration of NGF by intrathecal infusion was shown in the same study to prevent this injury-induced decrease in NF-M mRNA in neurons which express high affinity NGF receptors. The major role of the neurofilaments is maintenance of axonal calibre, which decreases proximal to the injury site after axotomy (Gold *et al.* 1991). Delivery of NGF to the proximal stump of an injured nerve partially prevents this decrease in axonal calibre and the associated decrease in neurofilament content (Gold *et al.* 1991), at least in groups of NGF-responsive nerve fibres. In addition, daily injection of NGF antiserum to normal adult rats causes a decrease in axonal calibre in the proximal axon of intact DRG neurons (Gold *et al.* 1991). Thus it is possible that one role of retrogradely transported NGF in the adult rat is the maintenance of axonal calibre via regulation of neurofilament synthesis, at least in certain classes of neuron.

Retrogradely transported NGF may also regulate the levels of its own receptors. Levels of mRNA for both the high and the low affinity NGF receptors in the L_5 DRG are also down-regulated after peripheral nerve transection and restored by exogenous NGF (Verge *et al.* 1992). Interestingly, exogenous NGF up-regulates mRNA for the low affinity receptor, but has been shown to up-regulate the mRNA for the high affinity NGF receptor only in injured sensory neurons (Verge *et al.* 1992).

The substance P content of cervical sensory ganglia was significantly increased 72 h after NGF was injected into the forepaw of adult rats (Goedert *et al.* 1981). It has since been shown that, although regenerating adult sensory neurons in culture do not require addition of NGF or BDNF to their culture medium for survival (Lindsay 1988) (they may produce autocrine BDNF), exogenous NGF or BDNF enhance neurite extension (Lindsay 1988) and NGF mediates increases in both the peptide content (Lindsay *et al.* 1989) and mRNA (Lindsay & Harmar 1989) for substance P and calcitonin gene-related peptide (CGRP). The rat and bovine promoter sequences of the preprotachykinin-A (PPT-A) gene contain regions which confer NGF responsiveness and are putative transcription binding sites (Gilchrist *et al.* 1992). This suggests that NGF can influence the binding of regulatory protein(s) to the promoter region of the PPT-A gene, directly stimulating and regulating transcription of substance P. Similar regulatory mechanisms for CGRP are under study (Watson & Latchman 1995; Watson *et al.* 1995).

NGF is also thought to play a role in nociception, subcutaneous injections of exogenous NGF causing mechanical and heat hyperalgesia (Lewin & Mendell 1993); this could cause problems in the event of therapeutic use of NGF. It has also been demonstrated that intradermal injection of NGF into the foot pad of adult rats leads to an increase in the rate of nociceptive fibre sprouting and evokes de novo sprouting (Diamond *et al.* 1992). In denervated skin there is also collateral sprouting from neighbouring undamaged nerve fibres. This is associated with an increase in NGF mRNA in the skin (Mearow *et al.* 1993). Treatment of rats with anti-NGF antibodies prevented denervation-induced sprouting (Diamond *et al.* 1992). These data suggest that altered nociception and collateral sprouting in disease states could be related to alterations in trophic support. This presents an overwhelming case for a physiological role for NGF in regulating expression of endoskeletal and transmitter-related genes in primary afferent neurons of the adult nervous system. By association the other neurotrophins probably complement this role for other neuron types and may be selective for other genes. It follows, therefore, that the expression and action of the neurotrophins could present a primary focus for failure in peripheral neuropathies.

2. REGULATION OF NEUROTROPHIN EXPRESSION

A range of molecules and second messengers stimulate NGF gene expression in a variety of cell types, including Schwann cells, glioma cell lines and fibroblasts (Carswell 1993), but in the intact adult system, the relevance of these mechanisms remains to be elucidated. However, impaired expression of neurotrophins might be counteracted pharmacologically as an alternative to replacement therapy. Of major interest is the ability of agonists stimulating adrenoceptors, coupled to adenylate cyclase, to up-regulate NGF synthesis and secretion (Mocchetti *et al.* 1989). Recently, cultured smooth muscle cells have been shown to increase NGF synthesis in response to contractile stimuli (Tuttle *et al.* 1993). It has also been demonstrated that NGF levels in the heart correlate with the density of sympathetic innervation (Shelton & Reichardt 1984), and that depolarizing stimuli increase NGF mRNA levels in cultured rat hippocampal neurons (Lu *et al.* 1991). These observations suggest that impulse activity of neurons may influence NGF secretion by the target organ.

This functional regulation of NGF synthesis in intact nerves may be supplanted or invigorated if the nerve axon is damaged. Sciatic nerve section increases NGF mRNA in fibroblasts and Schwann cells of both proximal and distal sections of the nerve (Heumann *et al.* 1987; Lindholm *et al.* 1988). This initial increase in NGF mRNA is believed to be due to the induction of immediate early genes such as c-fos (Heumann *et al.* 1991). Following this early boost in NGF mRNA, interleukin-1, released from macrophages, is thought to produce a more lasting increase in NGF synthesis (Heumann *et al.* 1991). This mechanism is not uniform for all trophic factors, in that BDNF expression is also up-regulated in response to sciatic nerve transection, but the time course of induction is much slower than

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for NGF, starting 3–4 days post-lesion and reaching maximal levels after 3–4 weeks (Meyer *et al.* 1992). In addition, interleukin-1 is without effect on BDNF mRNA levels in cultured nerve explants (Meyer *et al.* 1992).

3. PRODUCTION AND ACTION OF NGF IN DIABETES

Criteria for demonstration of the involvement of deficiency of a trophic factor in the aetiology of diabetic neuropathy are outlined below.

1. Deficient expression or retrograde transport of the neurotrophic factor must be demonstrated in tissues from diabetic models and diabetic patients.

2. Deficient expression of genes, which respond tonically to the neurotrophic factor, must be demonstrated in neurons in diabetes.

3. Deficient expression of these genes in diabetes should be corrected by administration of the appropriate neurotrophic factor or selective stimulation of its endogenous production.

4. Administration of the neurotrophic factor should attenuate functional neuronal deficits characteristic of diabetic neuropathy.

Decreased capture and retrograde transport in the sciatic nerve of labelled iodine administered by injection of iodinated NGF into the footpad was observed in diabetic rats many years ago (Jakobsen *et al.* 1981). There is also a report of reduced retrograde transport of iodinated NGF in ileal mesenteric nerves in diabetic rats (Schmidt *et al.* 1986). These observations imply that, even in the absence of any deficit in production of endogenous NGF in diabetes, a deficit in the amount delivered to neuron cell bodies might be expected. Subsequent observations further indicated that there were selective deficits in expression of endogenous NGF in different target tissues. Thus, in diabetic rats there are reduced levels of NGF in the submandibular gland, superior cervical ganglion and sciatic nerve (Hellweg & Hartung 1990; Hellweg *et al.* 1991). These reductions were attenuated or prevented by either insulin treatment or allogenic pancreatic islet transplantation (Hellweg *et al.* 1991). NGF levels have also been shown to be decreased in the serum of diabetic patients with peripheral neuropathy (Faradji & Sotelo 1990). The latter study demonstrated a significant correlation between serum NGF levels and motor nerve conduction velocity (MNCV). The importance of this finding is unclear and the correlation may be coincidental as NGF is not thought to act on motoneurons, nor is the relevance of serum levels established. Recent work in our laboratory has shown that, with increasing durations of diabetes, progressive reductions in NGF mRNA appear in different tissues. Leg muscles and the sciatic nerve show early reductions after just four weeks of diabetes (Fernyhough *et al.* 1993*a*, *b*), but there is no evidence of reduced NGF expression in foot skin until 12 weeks of diabetes (Fernyhough *et al.* 1992). It is clear, therefore, that diabetic rats show distinct reductions in expression of NGF in neuronal target tissues of the hindlimb.

We have made a detailed study of the retrograde transport of endogenous NGF in the sciatic nerve of diabetic rats, by measuring the amount of NGF-like immunoreactivity (NGF-LI) accumulating distal to a crush applied to the sciatic nerves of diabetic rats and the steady state amounts in the contralateral

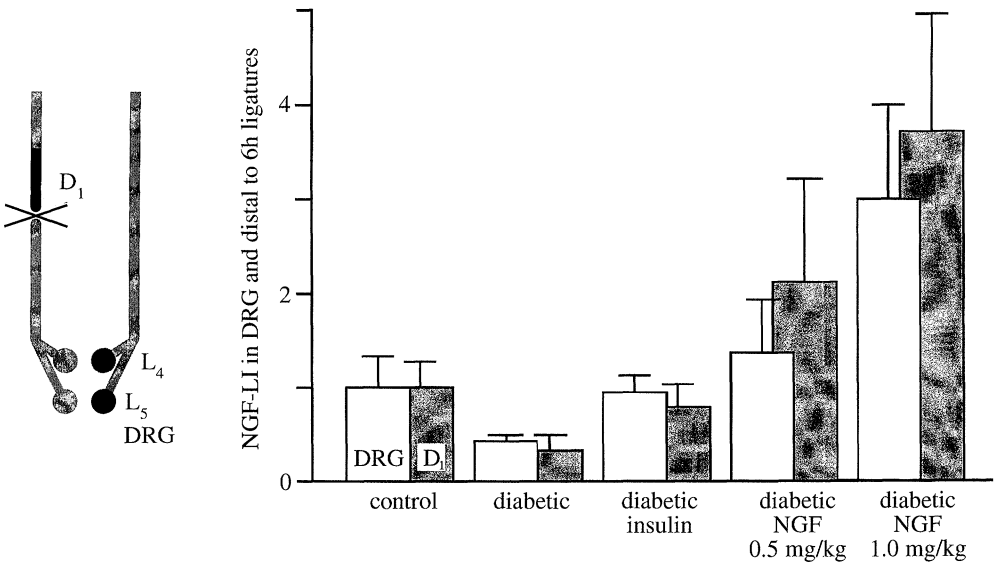

Figure 1. Retrograde transport of nerve growth factor in sciatic nerves of control and diabetic rats; effects of intensive insulin treatment or of administration of human recombinant NGF. The duration of diabetes was 8 weeks; treatments were initiated after 4 weeks and maintained thereafter. Details of insulin implants and NGF treatment are given elsewhere (Fernyhough *et al.* 1995). The drawing on the left indicates the tissue analysed. The D_1 segment was 1 cm sciatic nerve distal to a 6 h constricting ligature on the left sciatic nerve and the DRG were the $L_4 + L_5$ dorsal root ganglia from the contralateral side. NGF-like immunoreactivity levels in the diabetic tissues were referenced to control means as were the s.d. values for the control means. Mean data from untreated diabetic rats differed significantly ($p < 0.05$) from all other group means; means from the NGF 1.0 mg kg^{-1} group differed significantly ($p < 0.01$) from those of untreated and insulin-treated groups.

Table 1. *NGF mRNA and NGF-like immunoreactivity (NGF-LI) levels in soleus muscle; effects of insulin and of human recombinant NGF*

(NGF mRNA levels were quantified by Northern blotting as previously described (Fernyhough *et al.* 1994). Values are presented relative to control and adjusted to an internal recovery standard. NGF protein levels were determined using an ELISA kit obtained from Boehringer. Values are means ± s.d.)

	NGF mRNA	NGF-LI
	relative to control	pg mg^{-1} wet mass
control	1.0 ± 0.3 ($n = 5$)	0.92 ± 0.74 ($n = 6$)
diabetic	0.62 ± 0.14[a] ($n = 3$)	0.27 ± 0.1[b] ($n = 7$)
diabetic + insulin	0.87 ± 0.63 ($n = 5$)	1.11 ± 0.64 ($n = 6$)
diabetic + NGF (1.0 mg kg^{-1})	0.55 ± 0.17 ($n = 5$)	0.97 ± 0.54 ($n = 7$)

[a] $p < 0.05$ for diabetic *versus* control.
[b] $p < 0.05$ for diabetic *versus* all other groups (one-way ANOVA with Duncan's range tests).

(unligated) lumbar dorsal root ganglia. The study design also included a group of diabetic rats, whose diabetes was stringently controlled via subcutaneous insulin-delivery implants, and two groups of diabetic rats given subcutaneous injections of human recombinant NGF. The data are shown in figure 1. There were clear deficits in the amounts of NGF-LI accumulated distal to 6 h ligatures on the sciatic nerve and the amounts in the contralateral lumbar ($L_4 + L_5$) dorsal root ganglia were reduced in the untreated diabetic rats by a similar amount relative to controls. Insulin treatment normalized both the sciatic nerve distal accumulation and the lumbar ganglion content of NGF-LI and the diabetic rats treated with NGF showed dose-related increases over and above the untreated controls. This experiment shows a clear deficit in retrograde axonal transport of NGF and prevention of this deficit via maintenance of tight glycaemic control with insulin. The increases seen with NGF treatment demonstrate that exogenous human NGF gained access to neurons whose gene expression it might influence. This assertion is further supported by the demonstration that treatment with human NGF was without effect on the deficient expression of endogenous NGF in target tissues of the limb contralateral to the sciatic constriction. The data are presented in table 1, which shows a clear deficit in NGF mRNA in soleus muscle from untreated diabetic rats, a prevention of this deficit by insulin treatment, but no effect of NGF treatment. In contrast, the content of NGF-LI, which was also reduced in hindlimb muscle of diabetic rats, was increased not only by insulin treatment, but also by human NGF, showing that the exogenous NGF accessed target tissues. It is clear, therefore, that expression and retrograde transport of NGF is deficient in diabetic rats, satisfying the first criterion listed above. Furthermore, appropriate pharmacological intervention can surmount this deficit.

The second and third criteria we have established require demonstration of reduced expression in diabetes of genes tonically controlled by neurotrophins and reversal/prevention of this reduction by administration of the exogenous trophic factor. So far this has proved to be more straightforward for NGF than for the other neurotrophins because tonically regulated gene targets have not yet been identified for NT-3, NT-4 or BDNF in peripheral nerves, whereas the neuropeptides form clear targets for NGF (Lindsay & Harmar 1989; Lindsay *et al.* 1989). Deficient expression of substance P in lumbar primary afferent neurons has proved to be a reproducible deficit in diabetic rats (Robinson *et al.* 1987; Tomlinson *et al.* 1988) and this deficit extends to calcitonin gene-related peptide (CGRP) (Diemel *et al.* 1992). It was therefore logical to examine the possibility that these neuropeptide deficits were secondary to diminished stimulation by NGF in sensory neurons of diabetic rats and we examined the levels of mRNA for both peptides in lumbar dorsal root ganglia, correlating these with the levels of the peptides themselves in the sciatic nerves. This was done in control and diabetic rats, together with a diabetic group which had been treated with murine NGF. The full study is reported elsewhere (Diemel *et al.* 1994), but the findings with CGRP are illustrated as an example in figure 2, which shows good correlation between mRNA and peptide levels and indicates that the effects of diabetes and of NGF are probably mediated via alterations in mRNA levels. The changes in preprotachykinin mRNA and in substance P were similar (Diemel *et al.* 1994).

In a more extensive study, diabetic rats were treated with human recombinant NGF and dose-related effects were examined; also the levels of the neuropeptides were related to the levels of NGF in the same nerves and the effects of NGF were compared with those of intensive insulin treatment (for a full account see Fernyhough *et al.* 1995). The data are shown in table 2 and good correlation is evident between the levels of NGF and of the peptides, substance P and CGRP, though it appears that the highest dose of NGF used to treat the rats was supramaximal for increased CGRP expression.

It is clear that deficits in NGF expression develop in experimental diabetes and are accountable for reduced expression of gene targets for the neurotrophin. However, this does not explain the older observation of reduced capture and retrograde transport of exogenous labelled NGF (Jakobsen *et al.* 1981; Schmidt *et al.* 1986) referred to earlier in this account. More recently we have studied expression of the high-affinity NGF receptor, trkA by measurement of its mRNA in lumbar

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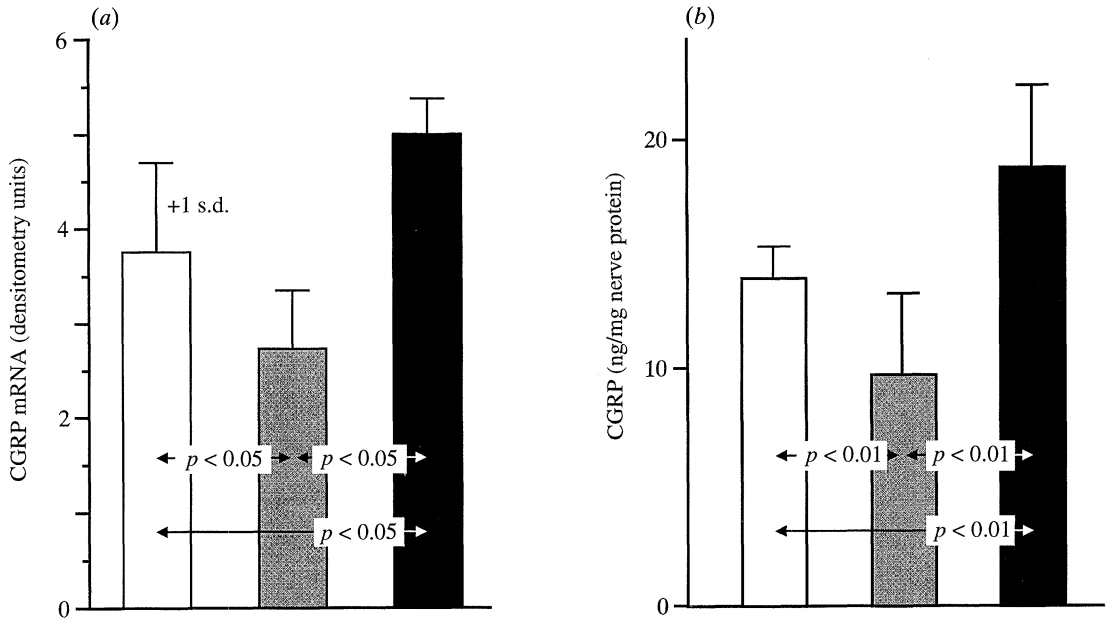

Figure 2. Expression of calcitonin gene-related peptide (CGRP) as mRNA in lumbar dorsal root ganglia and as peptide in sciatic nerve of the same rats, comparing controls (open columns), untreated diabetic rats (filled columns) and diabetic rats given murine 2.5S NGF (0.5 mg per rat s.c. 3 times per week for the final 3 weeks of 6 week period of diabetes; black columns). Data are mean + 1 s.d.; levels of significance are from one-way ANOVA with Duncan's multiple range tests.

Table 2. *Steady-state levels of NGF-LI, SP-LI and CGRP-LI in sciatic nerves of diabetic rats; effects of insulin and of treatment with human recombinant NGF*

(SP-LI and CGRP-LI were assayed in the same extracts; NGF-LI was measured in an adjacent segment of the same nerve. Data are mean ±s.d. Levels of significance are from one-way ANOVA with Duncan's multiple range tests. Homogeneity of variances (Cochran's and Bartlett-Box tests) were tested on $\log_{10}$-transformed data for NGF-LI and SP-LI.)

	NGF-LI	SP-LI	CGRP-LI
group/treatment (*n*)	pg cm^{-1} nerve	pg cm^{-1} nerve	ng cm^{-1} nerve
controls (8)	$72 \pm 20^{b,e}$	$83 \pm 19^{b,c}$	3.1 ± 0.7^{b}
diabetic untreated (10)	34 ± 12^{a}	55 ± 14^{a}	$1.8 \pm 0.6^{a,e}$
diabetic + insulin (8)	$62 \pm 18^{b,c}$	$87 \pm 12^{b,c}$	2.5 ± 0.3^{c}
diabetic + NGF at 0.2 mg kg^{-1} (7)	$107 \pm 51^{b,e}$	$111 \pm 46^{b,e}$	$4.2 \pm 1.7^{b,d}$
diabetic + NGF at 0.5 mg kg^{-1} (7)	$111 \pm 35^{b,d}$	$127 \pm 11^{b,d}$	$5.2 \pm 1.3^{b,d,f}$
diabetic + NGF at 1.0 mg kg^{-1} (8)	$198 \pm 106^{b,d,f}$	$144 \pm 39^{b,d,f}$	$5.2 \pm 1.3^{b,d,f}$

Levels of significance: for each column data superscripted [a] differs from data superscripted [b] at $p < 0.05$ and the same is true for [c] superscripts versus [d] superscripts and for [e] versus [f].

dorsal root ganglia of diabetic rats. This study has also incorporated the effects of axotomy in both control and diabetic animals and sub-groups of both were examined 1, 2 and 3 weeks after nerve section. The data are presented in figure 3, which illustrates the reduction in expression of trkA mRNA in the ganglia of the axotomized neurons, as has been observed by others (Verge *et al.* 1995). In addition it is clear that DRG levels of trkA mRNA were lower in diabetic rats in ganglia from both intact and axotomized sciatic nerves, than equivalent ganglia from control animals. Furthermore, this was evident at all three time points. Thus, the reduced retrograde transport of NGF in diabetic rats probably derives from a combination of reduced expression and impaired receptor-mediated capture. Besides changes in the synthesis of the trkA NGF receptor, the extracellular cleavage product of the low affinity NGF receptor (p75 NGFR-truncated) is found in plasma and urine and the urine concentration was increased in diabetic patients with neuropathy (Hruska *et al.* 1993). The same study demonstrated an increase in immunoreactivity for this low-affinity receptor in Schwann cells of neuropathic diabetic patients. Thus undisclosed mechanisms may provoke an increased turnover of the low-affinity NGF receptor. This may have functional consequences and additionally may offer useful diagnostic information via the change in urine.

Many molecules have been shown to regulate NGF expression such as noradrenaline, forskolin and 4-methylcatechol (for a review see Carswell 1993). Treatment of diabetic rats with 4-methylcatechol increased, but did not normalize, sciatic nerve NGF levels and was reported to prevent the decreased motor

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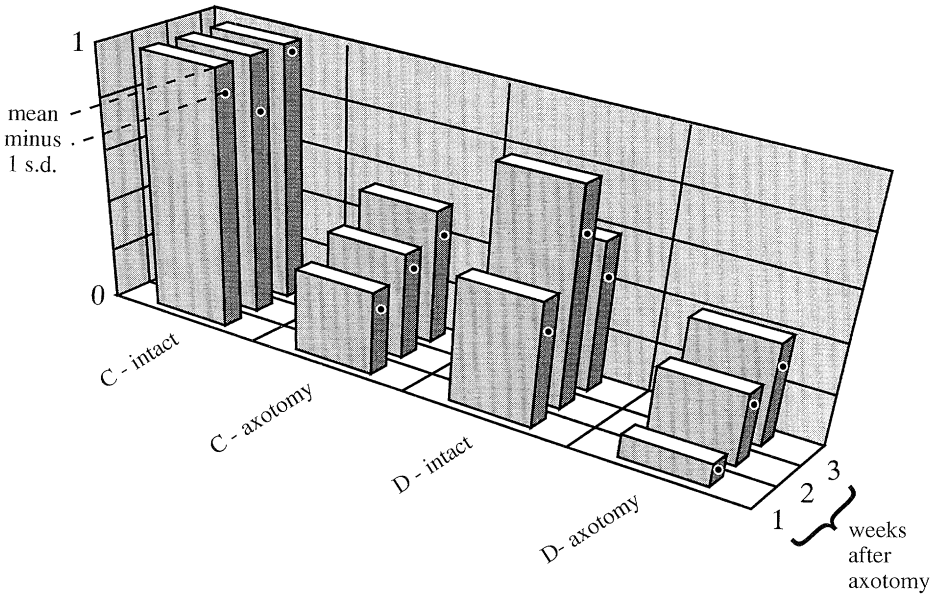

Figure 3. Three-dimensional bar chart showing expression of the mRNA for the high-affinity NGF receptor, trkA in dorsal root ganglia. Data are group means (minus 1 s.d. as indicated) derived from estimates of mRNA from unilateral pairs of dorsal root ganglia (L_4+L_5) from all sub-groups. Horizontal arrays show expression in ganglia from intact nerves and contralateral axotomized nerves (C = controls, D = diabetics) and vertical arrays show 1, 2 and 3 weeks post-axotomy.

nerve conduction velocity, which is a hallmark of short-term diabetes in rats (Hanaoka *et al.* 1992). This suggests that 4-methylcatechol can increase endogenous NGF *in vivo*. The effect on MNCV is unexpected as NGF is not thought to act on adult motoneurons: retrograde axonal transport labelling studies have shown that after injection of labelled neurotrophins at the site of a crush in the sciatic nerve, BDNF and NT-3 are found in motoneurons, whereas NGF is not (DiStefano *et al.* 1992). In addition, trkA mRNA is not detected in postnatal rat lumbar spinal cord, whereas trkB and trkC mRNA (which encode the high affinity receptors for BDNF and NT-3 respectively) are both present (Carroll *et al.* 1992). It is possible, however, that 4-methylcatechol is not specific for NGF and also stimulates BDNF and/or NT-3. The therapeutic potential for 4-methylcatechol and other NGF-inducing drugs in the treatment of diabetic neuropathy and perhaps other disorders of the nervous system warrants investigation.

4. OTHER NEUROTROPHINS

NGF could participate in some of the signs and symptoms of diabetic neuropathy, but it could not be responsible for all or even the major neurotrophic deficit. One of the most dangerous clinical manifestations of this condition is loss of protective sensation in the feet. This leads to insensible trauma and foot ulceration, with a very poor prognosis. The neurons responsible for this sensation are probably unresponsive to NGF and may be dependent upon support from other neurotrophic factors. NT-3 mRNA levels are reduced in leg muscle from diabetic rats (figure 4), but as stated above, it is difficult to make a systematic assessment of the tonic response to NT-3 because its gene targets are unidentified. We have, however, made

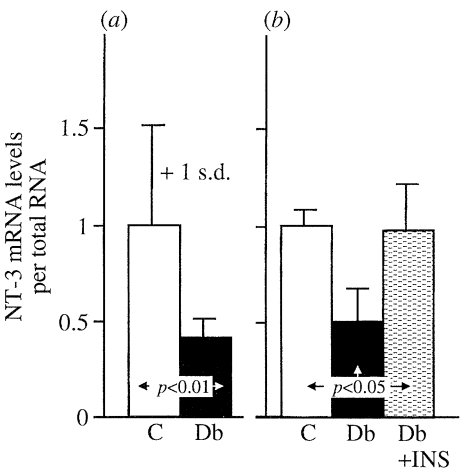

Figure 4. Levels of NT-3 mRNA (derived by northern blot hybridization) in soleus muscle from control (open columns) and diabetic (black columns) rats at (*a*) 6 and (*b*) 12 weeks duration of diabetes. Data from a group of 12 week diabetic rats given insulin is shown in hatched columns. Data are normalized to the control group mean for each duration of diabetes. Levels of significance were obtained by unpaired *t*-test (6 weeks) and by one-way ANOVA with Duncan's multiple range tests (12 weeks).

a pragmatic assessment of the possible influence of NT-3 in experimental diabetes. Diabetic rats develop reduced velocity of conduction in both motor and sensory fibres of the sciatic nerve; these can be measured as differences in motor and sensory latencies from the M wave and the H reflex, respectively. The data shown in table 3 illustrate the reductions in both motor and sensory velocity in rats with streptozotocin-induced diabetes of 12 weeks duration. The third group of rats were diabetic treated with human recombinant NT-3, given subcutaneously at the back of the neck at a dose of 1 mg kg^{-1} three times per week for the last four weeks of the period of diabetes. It is

Table 3. *Motor and sensory nerve conduction velocity deficits in diabetic rats; selective correction of the sensory deficit by NT-3*

(Data are mean ±s.d. Apparant differences were tested by one-way ANOVA with Duncan's multiple range tests. Homogeneity of variances (Cochran's and Bartlett-Box tests) were tested on $\log_{10}$-transformed data.)

	nerve conduction velocity (m s^1)	
	motor	sensory
untreated controls (11)	59.6 ± 9.5^{a}	66.1 ± 13.4^{c}
untreated diabetics (12)	45.1 ± 2.3^{b}	55.7 ± 11.7^{a}
diabetics + NT-3 at 1 mg kg^{-1} (9)	47.3 ± 5.8^{c}	72.9 ± 18.8^{b}

Levels of significance (coded by superscripts) are ^{a} *versus* ^{b}, $p < 0.01$ and ^{a} *versus* ^{c}, $p < 0.05$.

clear that the NT-3 treatment, which did not attenuate the hyperglycaemia of diabetes, had a powerful selective effect on sensory nerve conduction velocity. The groups of sensory fibres which elicit the H reflex would be expected to include those responsible for protective sensation in the foot, implying that NT-3 may be more instrumental than NGF in the development of important functional deficits in diabetic neuropathy and that normalization of its expression or pharmacological replacement may be an important therapeutic target.

5. CONCLUSIONS

In summary, therefore, impaired NGF trophic support develops in experimental diabetes and is manifest as reduced expression of the neuropeptides, substance P and CGRP in lumbar primary afferent neurons. The NGF deficit is partly a result of reduced production of the neurotrophin by target cells and partly a result of deficient expression of its high-affinity receptor, trkA, by the under-responding neurons. This impaired support from NGF could have deleterious effects on the sub-population of NGF-responsive neurons. Deficiencies in expression and response to NT-3 may have a greater influence on the loss of protective sensation in diabetic neuropathy. The ability of exogenous recombinant NGF and NT-3 to prevent at least some of the diabetes-associated metabolic changes, is an important step in understanding the pathogenesis of diabetic neuropathy and may have practical repercussions for the development of therapy or prophylaxis. The causation of these changes in neurotrophic support, as defined by links between their development and the biochemical imbalances of diabetes mellitus, remain to be elucidated.

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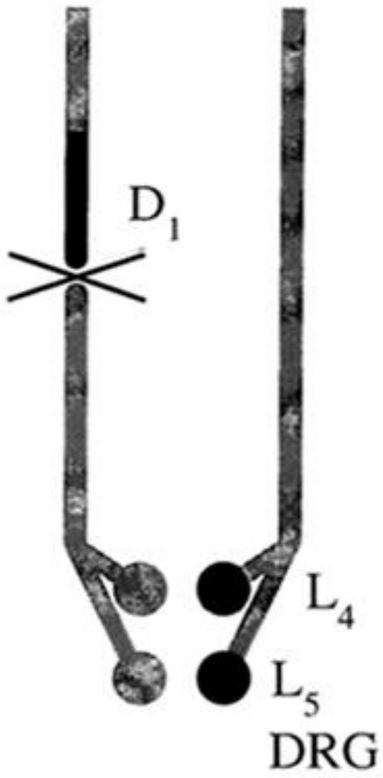
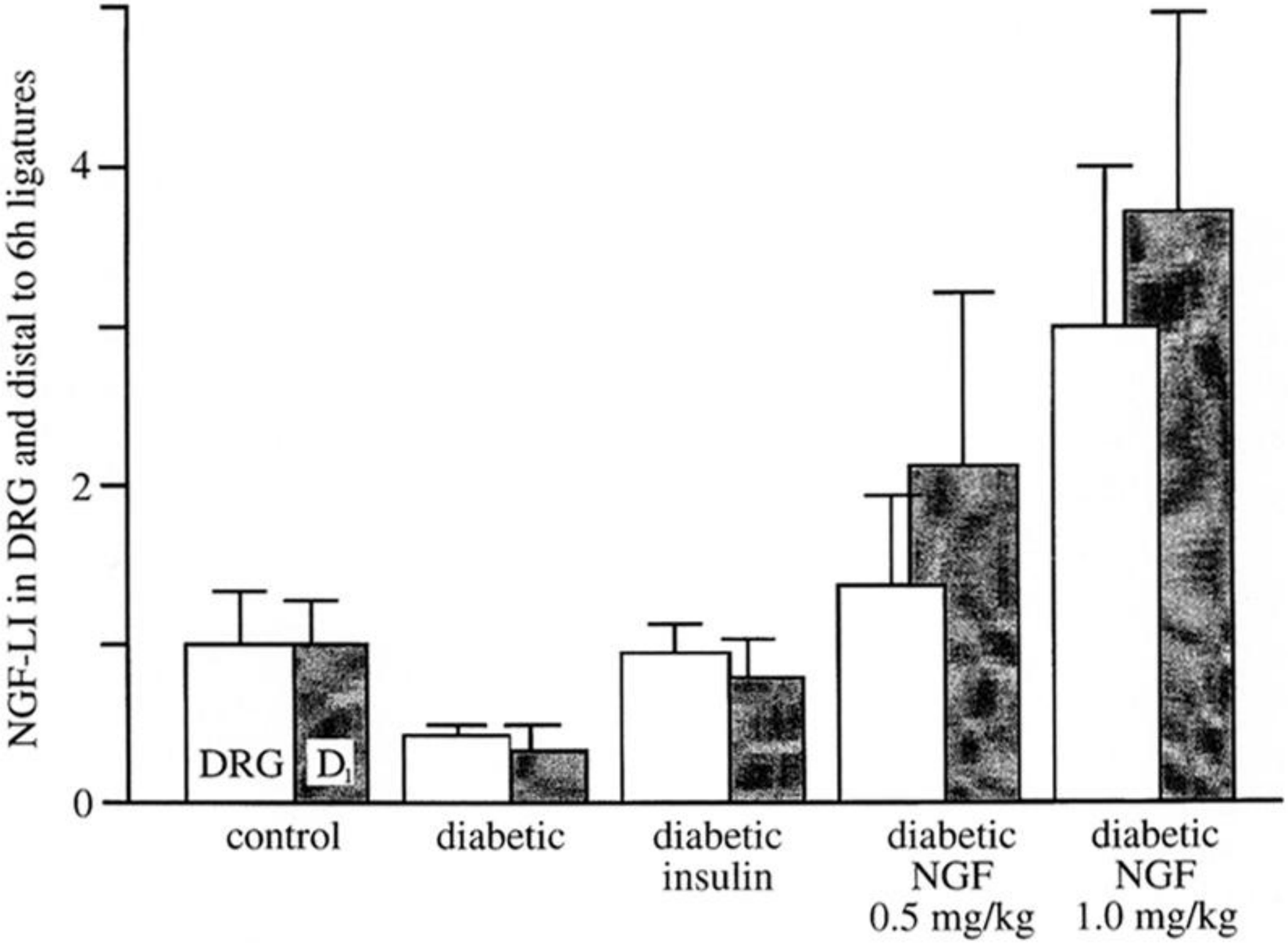
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES





gure 1. Retrograde transport of nerve growth factor in sciatic nerves of control and diabetic rats; effects of intensive sulin treatment or of administration of human recombinant NGF. The duration of diabetes was 8 weeks; treatments re initiated after 4 weeks and maintained thereafter. Details of insulin implants and NGF treatment are given ewhere (Fernyhough *et al.* 1995). The drawing on the left indicates the tissue analysed. The D_1 segment was 1 cm atic nerve distal to a 6 h constricting ligature on the left sciatic nerve and the DRG were the $L_4 + L_5$ dorsal root nglia from the contralateral side. NGF-like immunoreactivity levels in the diabetic tissues were referenced to ntrol means as were the s.d. values for the control means. Mean data from untreated diabetic rats differed nificantly ($p < 0.05$) from all other group means; means from the NGF 1.0 mg kg^{-1} group differed significantly (p 0.01) from those of untreated and insulin-treated groups.