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NT-3 modulates NPY expression in primary sensory neurons following peripheral nerve injury

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ABSTRACT

Peripheral nerve transection induces significant changes in neuropeptide expression and content in injured primary sensory neurons, possibly due to loss of target derived neurotrophic support. This study shows that neurotrophin-3 (NT-3) delivery to the injured nerve influences neuropeptide Y (NPY) expression within dorsal root ganglia (DRG) neurons. NT-3 was delivered by grafting impregnated fibronectin (500 ng/ml; NT group) in the axotomised sciatic nerve. Animals grafted with plain fibronectin mats (FN) or nerve grafts (NG) were used as controls. L4 and L5 DRG from operated and contralateral sides were harvested between 5 and 240 d. Using immunohistochemistry and computerised image analysis the percentage, diameter and optical density of neurons expressing calcitonin gene-related peptide (CGRP), substance P (SP), vasoactive intestinal peptide (VIP) and NPY were quantified. Sciatic nerve axotomy resulted in significant reduction in expression of CGRP and SP, and significant upregulation of VIP and NPY ($P < 0.05$ for ipsilateral vs contralateral DRG). By d 30, exogenous NT-3 and nerve graft attenuated the upregulation of NPY ($P < 0.05$ for NT and NG vs FN). However, NT-3 administration did not influence the expression of CGRP, SP or VIP. The mean cell diameter of NPY immunoreactive neurons was significantly smaller in the NT-3 group ($P < 0.05$ for NT vs FN and NG) suggesting a differential influence of NT-3 on larger neurons. The optical densities of NPY immunoreactive neurons of equal size were the same in each group at any time point, indicating that the neurons responding to NT-3 downregulate NPY expression to levels not detectable by immunohistochemistry. These results demonstrate that targeted administration of NT-3 regulates the phenotype of a NPY-immunoreactive neuronal subpopulation in the dorsal root ganglia, a further evidence of the trophic role of neurotrophins on primary sensory neurons.

Key words: Dorsal root ganglia; nerve regeneration; fibronectin; neuropeptides; trophic factors.

INTRODUCTION

Following axotomy of an adult peripheral sensory neuron the primary role of the neuron changes from one of neurotransmission to one of cell survival and regeneration. This change is accompanied by drastic changes in neuropeptide expression within the injured neurons. In particular, calcitonin gene related peptide (CGRP) (Dumoulin et al. 1992; Persson et al. 1995) and substance P (SP) (Tessler et al. 1985; Zhang et al. 1995) are downregulated while vasoactive intestinal peptide (VIP) (Nielsch & Keen, 1989; Kashiba et al. 1992) and neuropeptide tyrosine (NPY) (Wakisaka et al. 1991, 1993; Noguchi et al. 1993; Zhang et al. 1993; O'Hara et al. 1994) are upregulated.

The exact mechanisms which mediate these injury-induced changes are poorly understood but they may be due to a reduction in the normal retrograde transport of target-derived neurotrophic molecules (Raivich et al. 1991). This theory is supported by the finding that blockade of the normal axonal transport mechanism produces similar changes in neuropeptides (Knyihar-Csillik et al. 1991; Kashiba et al. 1992) and that when axons regenerate to their target organs neuropeptide levels return to normal (Bisby & Keen, 1986; O'Hara et al. 1994). Furthermore, the neuro-

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peptide profile of a neuron varies with peripheral target (Anand et al. 1983; McMahon et al. 1984; O'Brien et al. 1989), and alteration of the target organ of a neuron can change its subsequent peptide expression (McMahon et al. 1989), implying that target derived factors modulate neuronal phenotype.

Neurotrophins are a family of closely related growth factors including nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5) (Lindsay, 1996). The neurotrophins are now considered critical for the development, maintenance and regeneration of the nervous system. They are synthesised in peripheral target tissues, selectively taken up by the nerve terminals in a receptor-mediated manner and transported retrogradely to the neuronal soma where they provide trophic support and modulate phenotype (Lewin, 1996).

Adult sensory neurons cultured in the absence of exogenous neurotrophic factors can survive but lose specific phenotypic neuropeptide markers such as SP or CGRP, which are restored by the application of NGF (Lindsay & Harmar, 1989; Mulderry, 1994). Similarly, axotomy induced changes in SP and CGRP expression can be partially reversed by administering exogenous NGF to the proximal stump of the injured nerve (Fitzgerald et al. 1985; Wong & Oblinger, 1991; Zhang et al. 1995) or into the subarachnoid space (Verge et al. 1995). These data support a role for NGF in the maintenance of neuronal phenotype in the adult. As yet, however, there is little clear evidence that BDNF or NT-3 regulate the expression of phenotypic traits in mature DRG or other neurons.

Within lumbar dorsal root ganglia in rats, trkC, the high affinity receptor for NT-3 (Barbacid, 1994; Maness et al. 1994) is distributed mainly on a subpopulation of large and intermediate diameter neurons (McMahon et al. 1994; Kashiba et al. 1995). Studies with knockout mice (Ernfors et al. 1994; Klein et al. 1994; Tessarollo et al. 1994; Tojo et al. 1995), receptor blockade (Lefcort et al. 1996) and developmental experiments (Maisonpierre et al. 1990; Eriksson et al. 1994) provide further evidence that a subpopulation of large and intermediate neurons in the lumbar DRG are responsive to NT-3. Attempts have been made to correlate neuronal phenotype with neurotrophic sensitivity (Verge et al. 1989; Kashiba et al. 1996). NPY, unlike SP, CGRP and VIP, is found in a subpopulation of large and intermediate neurons following axotomy (Wakisaka et al. 1991, 1993; Noguchi et al. 1993; Zhang et al. 1993; O'Hara et al. 1994). As NT-3 primarily acts on similar sized neurons, NPY appears as an optimal candidate for investigating the potential role of NT-3 in regulating neuropeptide expression and phenotypic maintenance. There is evidence to suggest that delivery of NT-3 to the cut sciatic nerve or intrathecal administration of NGF diminishes the NPY upregulation in DRG as measured by radioimmunoassay (O'Hara et al. 1995) or by in situ hybridisation respectively (Verge et al. 1996). Limited immunostaining observations have been reported (O'Hara et al. 1995), but detailed information on quantitative immunohistochemistry is still lacking.

We have recently demonstrated that the local delivery of NT-3 to the proximal stump of transected sciatic nerves in rats enhances the rate and amount of nerve regeneration (Sterne et al. 1997*a*). Furthermore, this enhanced regeneration produces significant effects on motor target organ reinnervation possibly due to a direct effect on muscle afferent innervation (Sterne et al. 1997*b*). The purpose of this study was to investigate immunohistochemically the influence of the local delivery of NT-3 on the neuropeptide changes that normally accompany peripheral nerve injury.

MATERIALS AND METHODS

Surgery and tissues

Adult male Lewis rats were anaesthetised using 0.3 ml/kg of intramuscular Hypnorm (fentanyl citrate 0.315 mg/ml, fluanison 10 mg/ml; Janssen Pharmaceuticals) and 2.5 mg/kg of intraperitoneal diazepam (Phoenix Pharmaceuticals). The left sciatic nerve was exposed 5 mm distal to the sciatic notch via a gluteal muscle splitting incision. A 5 mm length of nerve was removed using sharp microsurgery scissors to produce a gap of 10 mm following retraction of the nerve ends. Using an operating microscope (Wild Heerbrugg), this gap was grafted either with fibronectin impregnated with 500 ng/ml NT-3 (NT) (Sterne et al. 1997*a*), plain fibronectin mats (FN) as controls, or reversed autologous nerve grafts (NG), as a further control.

During insertion, 14 mm long fibronectin mats were entubulated, the hydroscopic nature of the fibronectin causing them to expand, obliterating the lumen. The proximal and distal nerve stumps were drawn into either end of the entubulated mat to produce a resultant gap of 10 mm. For autologous nerve grafts a 1 cm length of sciatic nerve was removed, reversed and reanastomosed to bridge the resulting defect. All animal procedures were performed according to Home Office guidelines.

Six animals from each group were harvested at 5, 15, 30 and 240 d postoperatively. The animals were

anaesthetised as before and perfused, firstly using phosphate-buffered saline (PBS) followed by freshly prepared and filtered 4% paraformaldehyde solution in PBS. The thoracic and lumbar portions of the spinal cord were exposed and removed as a block together with the dorsal roots, dorsal root ganglia (DRG) and proximal sciatic nerves bilaterally. The tissues were postfixed in 4% paraformaldehyde solution for 6 h at room temperature, followed by several washes and storage at 4 °C in PBS-sucrose. Finally the paired L4 and L5 DRGs were removed by dissection under an operating microscope and operated and contralateral ganglia were blocked in pairs in O.C.T. (Tissue-Tek) ready for immunohistochemical processing.

Immunohistochemistry

From each pair of L4 and L5 DRGs serial 20 μm longitudinal sections were cut sequentially such that every 6th section was stained with the same antiserum. The sections were collected on Vectabond (Vector, UK) coated slides and air dried for 4 h at room temperature prior to immunostaining according to the indirect avidin-biotin complex (ABC) peroxidase nickel enhancement procedure (Shu et al. 1988). Polyclonal rabbit antibodies to SP (Affinity, diluted 1/5000), CGRP (Affinity, dil. 1/5000), VIP (Affinity, dil. 1/10000) and NPY (Affinity, dil. 1/4000) were used to identify known neuronal subpopulations. Each antiserum was applied to separate slides, and within each time point all sections were stained simultaneously, a known positive control being included in each batch of staining to ensure reliability of the technique. Addition of the relevant synthetic antigen (0.1 nmol/ml CGRP and NPY; 10 nmol/ml VIP; 20 nmol/ml SP) to the diluted antisera abolished immunostaining, confirming their specificity.

The sections were permeated in 0.2% phosphate-buffered Triton X-100 for 1 h; endogenous peroxidase was then inhibited by incubating the sections in 0.05% hydrogen peroxide in PBS for 30 min at room temperature. The sections were washed 3 times in plain PBS, then incubated in 3% goat serum (TCS Biologicals, UK) in phosphate buffered saline (pH 7.4) for 15 min at room temperature to block any background binding from the secondary labelled antibody, followed by 3 rinses in PBS. The sections were incubated with the first layer antibodies at 4 °C for 16–20 h. Following primary incubation the slides were washed by immersion in Tween (1:4000 in PBS) for 1 min to remove any excess unbound primary antibody followed by 3 washes in PBS before the biotinylated second layer goat antirabbit IgG (dil. 1/100 in PBS) was applied. The sections were incubated at room temperature for 60 min in a moist chamber, rinsed in PBS/Tween and 3 washes in PBS. Finally, peroxidase conjugated avidin–biotin complex (ABC, Vectabond, UK) was applied to the sections, which were incubated at room temperature for 60 min in a moist chamber. After PBS wash, the sections were equilibrated in 0.1 M sodium acetate buffer for 10 min and immersed in a developing solution (containing ammonium nickel sulphate, 3,3′-diaminobenzidine tetrachloride dihydrate, D-glucose and glucose oxidase in 0.1 M sodium acetate buffer pH 6.0) for 5–15 min. The reaction was terminated by removal from the activated solution and immersion in 0.1 M sodium acetate buffer pH 6.0. The sections were washed in PBS, rinsed in running tap water, dehydrated through ascending alcohols and cleared in xylene before being mounted in DPX microscopy mountant (BDH Laboratory Supplies, UK).

Alternate slides in each series were stained using cresyl violet in order to count the total number of neuronal cell bodies on tissue sections adjacent to those immunostained for neuropeptides (Terenghi et al. 1994). This count was then used to calculate the percentage expression of various neuropeptides (Coggeshall et al. 1994). The sections were immersed in filtered 0.1% (w/v) cresyl fast violet in distilled water for 5 min. The slides were then washed in running tap water and differentiated in 90% alcohol, taken to absolute alcohol and cleared in xylene before mounting in DPX.

Quantification

Quantification was carried out according to a previously published protocol (Terenghi et al. 1994). Using a dedicated software programme on an appropriately calibrated computerised image analysis system (Seescan Analytical Services, Cambridge, UK) and a ×25 microscope objective, 2 random sections from the ganglia of each animal for each stain were digitised and the images enhanced by background subtraction, thresholding and final editing. Only neurons whose nucleus was visible were counted. The number, size and optical density of immunoreactive neurons in injured and contralateral DRG were quantified by computerised image analysis. The corresponding, adjacent cresyl violet stained sections were similarly processed to provide a total neuronal count per section which was used in the subsequent calculation of percentage of expression of each neuropeptide.

Statistical analysis

Statistical analysis was carried out using a Sigmastat (Jandel Scientific, Germany) software package. The normality assumptions of the data were checked using Shapiro-Francia's W′ test and Bartlett's test was used to assure the equal variance assumption. A 1-way analysis of variance (ANOVA) was used to compare neuropeptide expression, optical densitometry and neuronal diameter in each group at each time point. Differences between individual groups were subsequently isolated by the use of an appropriate all pairwise multiple comparison procedure.

RESULTS

A mean total of 310 (±8) neurons stained with cresyl violet were counted on each longitudinal DRG section, with a total of about 4000 neurons counted per time period per group.

Neuropeptide Y

Immunoreactivity for NPY was not detectable in normal uninjured dorsal root ganglia but was upregulated following injury in each group (Fig. 1). NPY was expressed predominantly in intermediate and large neurons. Following nerve injury NPY became also detectable in a small proportion (< 1%) of neurons of the contralateral uninjured ganglia. In the FN group the proportion of neurons expressing NPY increased slowly to a maximum by d 15 which persisted to d 30 (Table 1). By 8 mo the proportion of neurons expressing NPY had declined considerably, but at least 6% of neurons in each group still contained strongly detectable levels of immunoreactivity. The addition of NT-3 significantly reduced

Table 1. *Mean percentage (±S.E.M.) of NPY-immunoreactive neurons within the dorsal root ganglia in each group at various time periods*

	Day 5	Day 15	Day 30	Day 240
NG%	11.66 (±0.8)	31.8 (±1.8)	20.1* (±3.2)	6.6 (±1.7)
NT%	9.74 (±3.1)	24.5 (±3.2)	19.2* (±1.5)	7.6 (±1.6)
FN%	14.4 (±3.7)	31.8 (±3.4)	34.2 (±3.4)	9.1 (±1.9)

Following sciatic nerve transection there was a gradual upregulation of NPY immunoreactivity in the injured neuronal cell bodies. The local delivery of NT-3 to the injured nerve after transection led to a generalised decrease of this upregulation which reached significant levels at d 30. * $P < 0.05$ for NT and NG vs FN, ANOVA, Tukey's test.

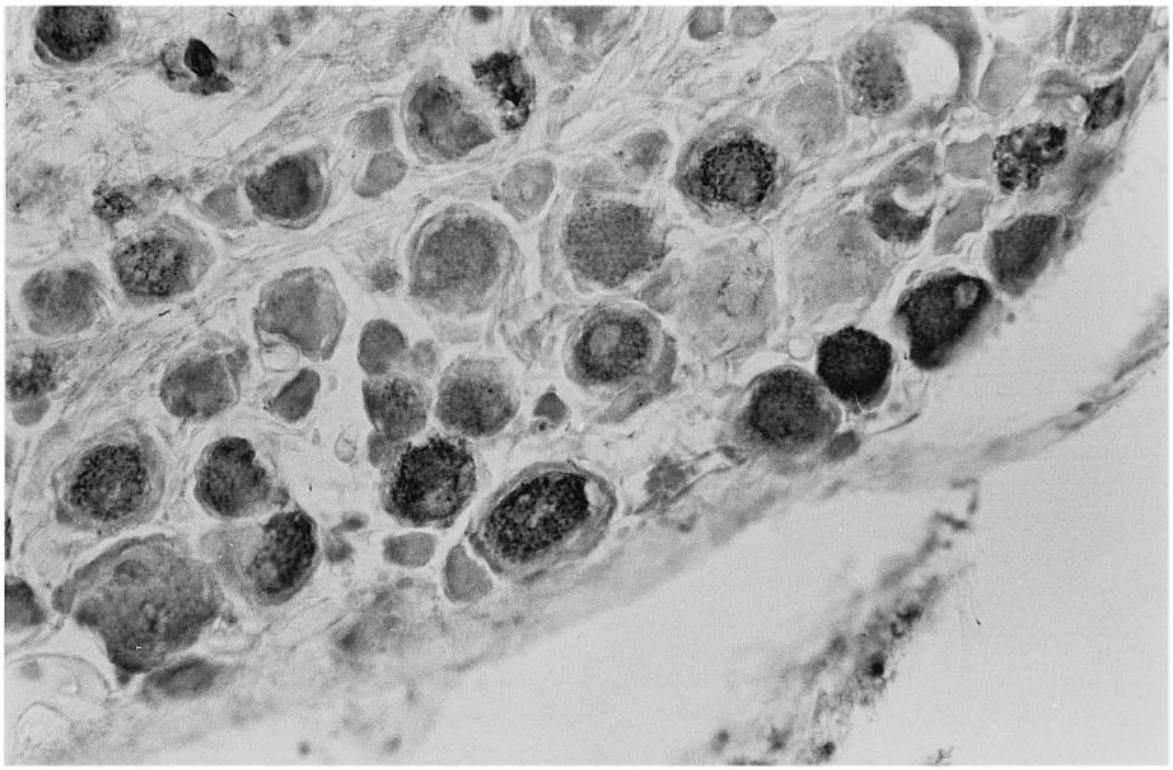

Fig. 1. Immunostaining of longitudinal 20 μm cryostat sections of L5 DRG at 15 d postoperatively. NPY-immunoreactive neurons became detectable ipsilaterally in large and intermediate sized neurons which showed a well defined punctate staining of the soma.

Table 2. *Mean size and optical density (±S.E.M.) of NPY immunoreactive neurons in each group at each time point*

	Day 5	Day 15	Day 30	Day 240
NG diameter (μm)	43.61 (±0.8)	41.68 (±0.44)	41.52 (±0.92)	37.81 (±0.54)
NT diameter (μm)	44.88 (±1.1)	38.31* (±0.75)	37.56* (±1.14)	38.17 (±0.63)
FN diameter (μm)	43.5 (±1.1)	41.24 (±0.95)	43.12 (±0.78)	38.89 (±0.62)
NG OD	0.28 (±0.01)	0.39 (±0.02)	0.26 (±0.02)	0.31 (±0.01)
NT OD	0.32 (±0.01)	0.45 (±0.02)	0.27 (±0.01)	0.25 (±0.01)
FN OD	0.26 (±0.01)	0.42 (±0.02)	0.25 (±0.01)	0.31 (±0.01)

At d 5 there was no obvious size difference in the groups but by d 15 the DRG in the NT group there were significantly smaller NPY immunoreactive neurons than in the other groups and this difference persisted until d 30. Optical density is expressed in arbitrary units. * $P < 0.05$ for NT vs NG and FN, ANOVA, Tukey's test.

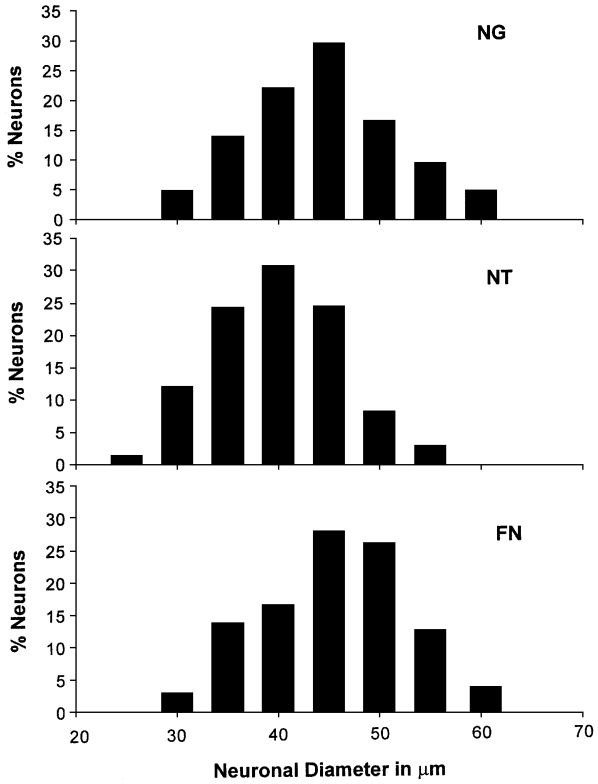

Fig. 2. Neuronal size distribution of NPY immunoreactive neurons for each group at d 30. There is a significant shift to a smaller size of NPY immunoreactive neurons in the NT group compared with the other 2 groups ($P < 0.001$; ANOVA, Tukey's test). The NG and FN groups do not differ significantly.

the upregulation in NPY expression. This down-regulating effect was evident at d 5 and 15 but failed to reach statistical significance at d 5 and 15, but by d 30 the proportion of neurons expressing NPY was significantly less in the NT group than in the FN group ($P < 0.05$, ANOVA, Tukey's test). For the NG and FN groups, there was no difference in the proportion of cells expressing NPY up to d 15 but by d 30 the level of NPY expression in the NG group had fallen significantly ($P < 0.05$ for NG vs FN, ANOVA, Tukey's test). By 8 mo there was no significant difference in the percentage of NPY-immunoreactive neurons between groups.

At d 30, the mean diameter of NPY-immunoreactive neurons was significantly less in the NT than in the FN or NG groups ($P < 0.05$, ANOVA, Tukey's test) (Table 2). The neuron size distribution was further examined (Fig. 2) demonstrating a differential shift from large to smaller sized NPY immunoreactive neurons in the NT group compared with FN and, less evidently, with NG groups.

The mean level of optical density, an indication of the level of NPY immunoreactivity within neurons, was found to be slightly higher in the NT group than in the FN and NG groups at the early time points but this failed to reach significance (Table 2). When this was further examined by comparing the optical densities according to neuronal size distribution (results not shown), it was noted that there were no clear significant differences between the groups.

Calcitonin gene-related peptide

In unoperated side ganglia, CGRP immunoreactivity was mainly expressed in small and some intermediate sized neurons (mean cell diameter 28 ± 1 μm) which accounted for 45 (±2)% of total neurons in the lumbar dorsal root ganglia. Five days after nerve injury, there was a significant generalised reduction in the number of CGRP-immunoreactive neurons to about 33 (±2)% ($P < 0.05$ for all groups, operated vs contralateral, ANOVA, Tukey's test) and this decrease continued to a minimum of 20 (±2)% by d 15. At d 30 there was still a significantly lower proportion of neurons expressing CGRP (26 ± 2%) in the ipsilateral DRGs of both groups with fibronectin grafts ($P < 0.05$ for FN and NT, operated vs contralateral). However by 30 d the proportion of neurons expressing CGRP in the NG group had returned to normal baseline levels of 41 (±4)% ($P > 0.05$ for NG, operated vs contralateral). By 8 mo, all injured ganglia contained the original proportions of CGRP staining neurons. There was a slight drop in the proportion of neurons in contralateral DRGs expressing CGRP at d 15 following nerve injury to 39

(±3)% but this did not reach statistical significance. NT-3 did not influence the neuronal cell size, optical density or level of expression of CGRP-containing neurons.

Substance P

Following nerve injury the number of neurons expressing SP immunoreactivity fell within the first 5 d from 23 (±2)% to an average of 10 (±1)% and remained at this level for at least 30 d. By 8 mo the number of SP staining neurons had returned to near baseline levels in all groups. SP was found to be expressed in small neurons of mean diameter 24.2 (±0.3) μm. Once again there was no evidence of any intergroup variation in the percentage expression, mean neuronal size or optical density. There was no detectable change in the proportion of neurons in contralateral DRGs expressing SP at any time point following nerve injury.

Vasoactive intestinal peptide

VIP was not detectable immunohistochemically in normal DRG and was not seen to be upregulated in any neurons in the contralateral uninjured DRG. Following nerve transection the number of neurons expressing VIP increased slowly from 5.5 (±1)% at d 5, up to 20 (±2)% at d 30. VIP immunoreactivity was no longer detectable in DRG neurons at 8 mo and was not seen to be upregulated in any neurons in the contralateral uninjured DRG. VIP was mainly expressed in small and some intermediate sized neurons of mean cell diameter 23.9 (±0.5) μm. NT-3 did not influence the number, size or level of expression of VIP-containing neurons. Similar results were seen in all nerve repair groups.

DISCUSSION

NPY immunoreactivity is normally undetectable in uninjured primary sensory neurons (Wakisaka et al. 1991; Noguchi et al. 1993) but, in the present study, following nerve injury NPY-immunoreactive neurons appeared progressively over the first 5 d and their percentage reached a peak by d 15, consistent with previous reports of the time scale of NPY upregulation (Wakisaka et al. 1991; Zhang et al. 1993, O'Hara et al. 1995; Verge et al. 1996). NPY immunoreactivity was found mostly in intermediate and large neurons, again in agreement with previous findings (Wakisaka et al. 1991, 1993; Noguchi et al. 1993; Zhang et al. 1993; O'Hara et al. 1994), and a few NPY-immunoreactive neurons, numbering less than 1%, appeared in the contralateral uninjured DRG (O'Hara et al. 1994). By 8 mo there was still a small percentage of cells in each group expressing NPY. Lack of axonal regeneration is generally associated with a continued upregulation of NPY, whilst crush injury is associated with a progressive decrease in NPY expression coincident with appropriate target organ reinnervation (O'Hara et al. 1994). Previously, we have been able to show that exogenous administration of NT-3 improves nerve regeneration (Sterne et al. 1997*a*), and that regenerating axons reinnervated a motor target organ (Sterne et al. 1997*b*). The reinnervation process was evident later than 30 d postoperatively, and it might have contributed partially to the downregulation of NPY seen at later time points of this study. It is also possible that the prolonged low percentage of NPY expression seen in this study may be the result of failed regeneration of a few neurons or reinnervation of an inappropriate target organ. Interestingly, this was observed in all experimental groups, which would exclude a specific group effect.

The local delivery of NT-3 to injured sciatic nerves appears to reverse partially the NPY upregulation that ensues within the neurons of the lumbar dorsal root ganglia, with a 44% reduction in the number of neurons expressing NPY. It cannot be completely excluded that this might be a consequence of the effect of NT-3 on regeneration rather than its direct effect on DRG neurons. However, the outcome appears to be specific for NPY-immunoreactive neurons, as administration of this neurotrophin does not affect the regulation of CGRP, SP or VIP following nerve injury and regeneration. Furthermore, plain fibronectin nerve repair facilitated nerve regeneration, but did not appear to influence the time course regulation of any investigated neuropeptides. Nerve graft repair attenuated the expression of NPY-immunoreactive neurons, but also restored the expression of CGRP immunoreactivity. It is likely that this wider spectrum of influence may be due to the presence of dedifferentiated Schwann cells in the graft, which are known to produce different growth factors, including NGF which regulates the expression of CGRP in primary sensory neurons (Lindsay & Harmar, 1989).

NGF and NT-3 are known to bind to specific high affinity receptors trkA and trkC respectively (Barbacid, 1994; Maness et al. 1994), although some degree of cross-reactivity of NT-3 with trkA and trkB has been reported (Rydén & Ibáñez, 1996). In our study, the exogenous delivery of NT-3 did not appear to affect the CGRP-containing neuronal cells, which also display trkA receptor (McMahon et al. 1994), at

least sufficiently for this effect to be detected by our investigative methods. Interestingly, nerve grafting appeared to have an effect on NPY-immunoreactive neurons. Because of the nature of the experiment, it was not possible to determine which of the growth factors produced by the Schwann cells in the grafted nerve might have had an effect on the NPY-containing cells, but it is tempting to speculate that some degree of cross-reactivity might have happened. This would not be surprising in view of the reports showing an overlapping in the distribution of different neurotrophic factor receptors in DRG neurons (McMahon et al. 1994; Michael et al. 1997).

Our results on NPY are consistent with previous evidence from radioimmunoassay studies which revealed a 36% diminution in NPY content of lumbar DRG following NT-3 application to the proximal nerve stump (O'Hara et al. 1995). Similarly, NPY mRNA upregulation following nerve injury is mitigated by intrathecal infusion of NGF (Verge et al. 1996). In this study we were able to demonstrate that the downregulatory influence of NT-3 was affecting differentially the NPY upregulation in large neurons with a resulting shift of the remaining NPY-immunoreactive neurons to a subpopulation of significantly smaller cells. Optical density measurements revealed that, despite the size shift, the NPY-immunoreactive neurons showed similar values to their untreated counterparts when matched for size. These results are in apparent contradiction with those previously reported, where the shift to a smaller population of NPY immunoreactive neurons following NT-3 administration was associated with decreased NPY immunofluorescent staining (O'Hara et al. 1995). However, this was a subjective assessment not validated by morphological quantification and impossible to assess for a subset of neurons by radioimmunoassay.

The functional significance of these alterations in neuropeptides is not fully understood. It has been proposed that following nerve injury neurotransmitters are downregulated and survival factors are upregulated (Barron, 1983), and that these chemical changes serve to adapt the neurons to the consequences of a nerve lesion, thus improving the conditions for survival and regeneration whilst protecting against other deleterious effects, such as lesion pain (Hökfelt et al. 1993). In adults NT-3 is normally delivered to neurons by retrograde axonal transport from the peripheral target tissues (DiStefano et al. 1992) and in view of our and previous results it is tempting to speculate that this NT-3 supply may keep the level of NPY expression low, as evidenced by the fact that NPY is not normally detectable in adult primary sensory neurons (Noguchi et al. 1993; Zhang et al. 1993). Following nerve transection there is an interruption in the supply of, amongst others, target tissue derived NT-3, which might lead to the appearance of NPY immunoreactivity. However, it has also been reported that NGF administered intrathecally can reduce the upregulation of NPY mRNA in lumbar DRGs, possibly acting either via trkA receptors on the neuronal cell body or by axonal uptake at its central processes (Verge et al. 1995).

The upregulation of NPY may subserve a role in a regulatory autocrine or paracrine feedback mechanism on primary sensory neurons expressing NPY receptors (Walker et al. 1988; Kar & Quirion, 1992; Zhang et al. 1994). NPY has a strong inhibitory mode of action; in particular it inhibits SP (Walker et al. 1988; Duggan et al. 1991) and CGRP release (Franco-Cereceda et al. 1992). Also the intrathecal administration of NPY has a depressive effect on the spinal nociceptive flexor reflex (Hua et al. 1991), indicating a role for this peptide in modulating pathological neuronal activity following nerve injury.

In conclusion, this study provides further evidence that neurotrophins differentially regulate neuropeptide expression in primary sensory neurons of the adult nervous system and in particular that NT-3 might regulate the expression of NPY in a subset of neurons in the lumbar dorsal root ganglia.

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