Published in final edited form as:

Brain Res. 2009 February 19; 1255: 1–8. doi:10.1016/j.brainres.2008.11.090.

Changes in NGF and NT-3 protein species in the superior cervical ganglion following axotomy of postganglionic axons

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Abstract

Mature sympathetic neurons in the superior cervical ganglion (SCG) are regulated by target-derived neurotrophins such as nerve growth factor (NGF) and neurotrophin-3 (NT-3). High molecular weight NGF species and mature NT-3 are the predominant NGF and NT-3 protein isoforms in the SCG, yet it is unknown whether the presence of these species is dependent on intact connection with the target tissues. In an attempt to determine the role of peripheral targets in regulating the neurotrophin species found in the SCG, we investigated the NGF and NT-3 protein species present in the SCG following axotomy (transection) or injury of the post-ganglionic axons. Following a 7 day axotomy, the 22–24 kDa NGF species and the mature 14 kDa NT-3 species in the SCG were significantly reduced by 99% and 66% respectively, suggesting that intact connection with the target is necessary for the expression of these protein species. As expected, tyrosine hydroxylase (TH) protein in the SCG was significantly reduced by 80% at 7 days following axotomy. In order to distinguish between the effects of injury and loss of target connectivity, the SCG was examined following compression injury to the post-ganglionic nerves. Following injury, no reduction in the 22–24 kDa NGF or 14 kDa mature NT-3 species was observed in the SCG. TH protein was slightly, yet significantly, decreased in the SCG following injury. The findings of this study suggest that the presence of the 22–24 kDa NGF and mature 14 kDa NT-3 species in the SCG is dependent on connection with peripheral targets and may influence, at least in part, TH protein expression in adult sympathetic neurons.

Keywords

sympathetic regulation; proneurotrophin; neurotrophin regulation; compression injury; western analysis

1. Introduction

The survival and maintenance of sympathetic and sensory neurons are regulated by neurotrophins, a family of target-derived neurotrophic proteins utilized by the responsive innervating neurons. Two such neurotrophins used by sympathetic and some sensory neurons are nerve growth factor (NGF) and neurotrophin-3 (NT-3). The classic neurotrophin hypothesis is based on the idea that innervating neurons compete for a finite amount of ‘mature’ neurotrophin provided by the target (Korsching and Thoenen 1985; Zhou et al., 1997). However, with regard to NGF, there appears to be relatively little mature NGF (13 kDa species)

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in most peripheral and central nervous tissues and high molecular weight (MW) species other than mature NGF predominate (Reinshagen et al., 2000; Fahnestock et al., 2001; Bierl et al., 2005; Randolph et al., 2007). In contrast, the mature form of NT-3 (approximately 14 kDa) is abundant in rat peripheral neurons as well as their targets (Bierl and Isaacson 2007; Randolph et al. 2007). Interestingly, high MW NGF molecules are detected in peripheral ganglia containing neurons thought to derive NGF from their targets by retrograde transport (Korsching and Thoenen 1985). Whether these high MW species are target-derived and/or dependent on signals from the target is unknown.

The presence of high MW precursor molecules in peripheral ganglia such as the superior cervical ganglion (SCG) and trigeminal ganglion (Randolph et al., 2007) suggests the possibility that NGF biosynthesis takes place at the level of the neuronal cell body. Indeed, we, as well as others, have evidence of both NGF (Hasan et al., 2003; Bierl and Isaacson 2007) and NT-3 (Bierl and Isaacson 2007) mRNA transcripts in the SCG. Though a non-neuronal origin for these neurotrophin transcripts is possible, Hasan and colleagues (2003) used *in situ* hybridization to show NGF mRNA within SCG cell bodies, providing evidence that SCG cell bodies are capable of NGF biosynthesis. Therefore, it is also possible that at least some of the neurotrophin species present in the SCG are intrinsic to the ganglion.

In order to distinguish between the two possible origins for NGF and NT-3 protein species in the SCG and to determine which neurotrophin species were dependent on intact connection with peripheral targets, the SCG cell bodies were disconnected from their peripheral targets by transection of the post-ganglionic nerves (axotomy). In order to determine the effects of injury, in a different set of animals, the postganglionic nerves were subjected to compression injury and changes in the SCG were compared to those obtained following axotomy.

2. Results

Mature NGF (13–16 kDa) was not detected in the adult rat SCG from controls or treated animals, yet high MW NGF species (22–24, 34, 55, 73–75 kDa) were relatively abundant (Fig. 1). The only tissue in which the mature NGF species was abundant was the male mouse submandibular gland (Figs. 1B,D). These results are consistent with previous findings from our laboratory (Bierl et al., 2005; Bierl and Isaacson 2007; Randolph et al. 2007) showing that mature NGF is only a minor NGF species in most peripheral tissues.

All ganglia used in the axotomy group were taken from animals showing obvious ptosis. In addition, post-surgical examination of each SCG analyzed revealed successful transection of the post-ganglionic nerves. Following both 24 hour and 7 day axotomy, changes in the 22–24 kDa and 75 kDa species were the most pronounced and consistently observed across the treatments. The 22–24 kDa NGF species was decreased by 85% at 24 hours after axotomy and was below the level of detection at the 7 day timepoint (Figs. 1, 2). In addition, the 73–75 kDa isoform was significantly increased by 96% at 24 hours following axotomy, and remained slightly increased by 28% at 7 days following the axotomy procedure.

In order to assess whether the changes in NGF species observed in the SCG might be a response to injury associated with the axotomy rather than disconnection with peripheral targets, the SCG was examined 24 hours or 7 days following compression injury to the post-ganglionic nerves (Figs. 1–4). No cases used in the crush treatment group showed any signs of transection as determined by post-sacrifice assessment. The appearance of ptosis was somewhat variable and ranged from mild-moderate ptosis to no noticeable change in the eyelid appearance. At both the 24 hour and 7 day post-injury timepoints, the 22–24 kDa NGF species was similar to control levels or slightly decreased, respectively, suggesting that the loss of this NGF species observed following axotomy may be a response to disconnection with the peripheral target

rather than a response to injury. Similar to that observed following 24 hour axotomy, the 73–75 kDa NGF species was significantly increased following a 24 hour injury, but levels of this species dropped to control levels at 7 days post-injury.

NT-3 western analysis revealed that, in contrast to the lack of mature NGF in the SCG, the mature NT-3 species (approximately 14 kDa) was abundant in the SCG (Fig. 3). Following axotomy, mature NT-3 was significantly decreased at both the 24 hour (58%) and 7 day (66%) timepoints. No overall reduction in NT-3 was observed following injury at either the 24 hour or 7 day timepoints. However, the response was somewhat variable and seemed to parallel the extent of injury, with the most injured ganglia (as indicated by degree of ptosis) generally showing more reductions in mature NT-3.

TH protein in the SCG was assessed in order to examine the effects of axotomy and injury. Consistent with previous studies (Sun and Zigmond, 1996), TH protein in the SCG was significantly decreased following 24 hour (53%) and 7 day axotomy (80%) (Fig. 4). Following injury, the decrease was not as dramatic as that observed following axotomy, though TH protein was significantly decreased at both 24 hours (25%) and 7 days (25%) post injury (Fig. 4).

3. Discussion

Neurotrophin biosynthesis is typically thought to take place in target tissues (Korsching and Thoenen 1985; Edwards et al., 1986; Rattenholl et al., 2001) and, until recently, it has been widely accepted that the mature species of NGF and NT-3 were the primary species released from peripheral targets and subsequently responsible for the biological response observed in the innervating neurons (Friedman and Greene 1999). Yet, we, along with others, have found that high MW NGF species other than ‘mature’ NGF are present in both central and peripheral tissues (Reinshagen et al., 2000; Fahnstock et al., 2001; Bierl et al., 2005; Bierl and Isaacson 2007). Interestingly, these precursor molecules also are found in peripheral neurons which are traditionally known to derive neurotrophin from their targets for transport to the cell body. Indeed, we (Bierl and Isaacson 2007), as well as others (Hasan et al., 2003) have evidence of NGF transcripts in the SCG and trigeminal ganglion. Though a non-neuronal origin of these neurotrophin transcripts is possible, Hasan and colleagues detected NGF mRNA in the SCG cell bodies using *in situ* hybridization (Hasan et al., 2003), providing evidence of NGF biosynthesis in peripheral neurons.

In the present study, we attempted to determine whether the presence of any of the NGF or NT-3 protein species in the SCG was dependent on intact connection with peripheral targets by examining SCG tissue lysates following transection of the post-ganglionic neurons. As expected, the mature (13–16 kDa) species of NGF was not observed in the SCG from control or treated tissues. Yet mature NT-3 was prevalent. We have shown previously that high MW NGF species were the predominant NGF forms in the SCG (Bierl et al., 2005; Bierl and Isaacson 2007) while the mature form of NT-3 was abundant (Bierl and Isaacson 2007; Randolph et al., 2007). Following axotomy, the 22–24 kDa NGF species and the mature NT-3 protein species were decreased in the SCG at both timepoints examined, suggesting that disconnection with the peripheral targets resulted in the loss of these neurotrophin species in the SCG. This finding supports the idea that these species may represent retrogradely transported species or that their presence in the SCG may be regulated by target-derived signals. Consistent with this idea, Zhou and colleagues (1997) provided evidence that NT-3 in the SCG can be derived from peripheral targets. Our results extend these findings and reveal the specific neurotrophin species in the SCG that may be derived from or regulated by the peripheral targets.

While it is possible that the presence of the 22–24 kDa NGF species and the mature NT-3 species is merely regulated by target-derived signals and not the result of transport from the

target, there is support that the 22–24 kDa NGF species present in the SCG is target derived. We recently reported an increase in the 22–24 kDa NGF species in the trigeminal ganglion following a two week intracerebroventricular administration of NGF peptide (Randolph et al. 2007). This finding parallels our previous ELISA findings showing increased NGF levels in the trigeminal ganglion following *in vivo* NGF administration (Shoemaker et al., 2002). Thus, it appears that the 22–24 kDa NGF species may not represent a precursor form of NGF such as the 32 kDa proNGF species, but rather a retrogradely transported processed form of the ‘mature’ NGF peptide that accumulated in the trigeminal ganglion following NGF administration.

Because the 22–24 NGF and mature NT-3 species might be altered by the release of soluble factors from the injured nerves or by some other response to injury caused by the axotomy procedure, the SCG was examined following injury, but not transection, of the post-ganglionic nerves. Though nerve crush in the past has been used as a means to cause degeneration while leaving the glial pathways for neuronal regeneration intact, it was our intent in the present study to injure the postganglionic axons but to cause minimal degeneration. Based on the absence of ptosis or the presence of only mild ptosis in our injury cases, we believe that the postganglionic axons remained relatively intact. In each injury case examined for NGF, NT-3 and TH, we observed an increase in ATF-3 protein in the SCG (Isaacson, unpublished observation), indicating that the crush procedure used in our study resulted in injury to the post-ganglionic axons, even when ptosis was only mildly detectable. We found that the effects of crush injury on the 22–24 kDa NGF and mature NT-3 species were minimal. Indeed, these presence of these neurotrophin species in the SCG appeared to be relatively unaffected by the injury, suggesting that the transport of these protein species or of signals that regulate their presence may not be as affected by the injury procedure when compared to complete transection (axotomy).

There are reports that compression injury can alter axonal transport (Dahlin et al., 1986; Dahlin et al., 1987; Zhou et al., 1997), yet the parameters in those studies were quite different from those used in the present study. In agreement with the results of the current study, which suggest that transport may occur across the injury site, no loss of axonal transport of tubulin was detected at 2 days or 7 days following a 2 hour compression injury (Dahlin et al., 1993). Others also have shown the transport of protein molecules along injured axons. For example, synapsin I was shown to be anterogradely transported across injured sciatic nerve after a 24 hour ligation injury (Kwon et al., 2000).

However, an alternative explanation for the presence of these neurotrophin species in the SCG following injury of the post-ganglionic axons may be that the injured, but not transected, post-ganglionic axons either produced these species or derived these species (or a signal that regulates their presence in the SCG) from non-neuronal cells and/or tissues surrounding the injury site. Indeed, p75 immunoreactivity was differentially expressed in the facial nucleus following either crush (p75 was present) or axotomy (p 75 was not present) of the facial nerve (Bussmann and Sofroniew, 1999) and it was hypothesized that a retrograde signal derived from non-neuronal cells at the injury site may have contributed to the upregulation of p75 in the facial nucleus following crush but not axotomy treatment. Similarly, it appears that, in our study, injury and axotomy of post-ganglionic nerves produce different responses in the SCG parent neurons.

The reduction in TH protein in the SCG following axotomy is consistent with previous studies showing a decrease in TH mRNA after disconnection of the SCG with peripheral targets (Sun and Zigmond 1996). In that study, the lowest level of TH mRNA was observed by day 3 but recovered to control levels by 2 months. Because TH protein in sympathetic neurons is selectively induced by NGF (Thoenen et al., 1971), we conclude that the axotomy procedure results in the loss of a target-derived signal (and/or neurotrophin species) that may be necessary

for proper TH regulation. The findings of the present study support the idea that the necessary signal may involve the 22–24 kDa NGF species or the mature NT-3 species that is either derived from the target or regulated by a target derived signal. It is possible that the injury procedure resulted in some degeneration, and this may explain the slight loss of TH protein in the injured cases. Yet, compared to axotomy, TH protein expression in the SCG was only mildly affected following injury, a finding that is paralleled by the minimal changes in the 22–24 kDa NGF and mature NT-3 species. These results further support the idea that the presence of these neurotrophin species can influence TH activity in the SCG.

The 73–75 kDa NGF species showed a dramatic increase in the SCG at the 24 hour timepoint following both axotomy and injury, yet levels were similar to control values (or relatively close to control values in the case of axotomy) after 7 days. The exact nature of the 73–75 kDa NGF species is unclear, but this species represents the primary NGF form observed in the hair follicle (Peters et al., 2006), and there is evidence that the presence of this NGF species may influence hair development (Yardley et al., 2000). In the present study, the functional significance of the observed plasticity in the 73–75 kDa NGF species is unknown. Yet, because the increase in this species was observed following both axotomy and injury, particularly at the 24 hour timepoint, changes in the 73–75 kDa species may represent a response to injury. However, anterograde transport and secretion of at least some NGF precursor molecules have been reported (Hasan et al., 2003), so it is possible that the increase in the 73–75 kDa species in the SCG following axotomy and/or injury also could result from an accumulation in the ganglion due to a reduction in anterograde transport. Also, an accumulation of this high MW species could result from a reduction in any target-derived signals that regulate processing of high MW precursors to smaller protein species.

Few studies have examined NGF levels in the SCG following axotomy. Using ELISA, Nagata and colleagues (1987) showed a decrease in total NGF protein in the rat SCG following both 24 hour and 7 day axotomy. Likewise, in another NGF ELISA study, a significant decrease in NGF in the mouse SCG was observed following a 7 day axotomy (Shoemaker et al., 2006). Though it is not clear exactly which NGF species were actually measured in these ELISA experiments, the decreases may be the result of reductions in the 22–24 kDa NGF species. Alternatively, in a western analysis, Hasan and colleagues (2003) reported a slight increase in the mature forms with a decrease in high MW forms in the SCG following axotomy, though no quantitative methods were shown.

In summary, the results of the present study provide evidence that the presence of the 22–24 kDa NGF species, as well as the mature NT-3 species in the SCG is dependent on intact connection with peripheral targets and may result from retrograde transport and/or a retrograde signal provided by the target tissues. In addition, the presence of these neurotrophin species appears to be sufficient to regulate TH expression in the SCG. Further, we provide evidence that transection and injury of the post-ganglionic axons produce different changes in neurotrophin and TH protein expression in the SCG.

4. Experimental Procedure

Animals

Young adult (3 months of age) female Sprague Dawley (Harlan Labs, Indianapolis, IN) rats were housed in the Miami University Animal Facilities in a 12:12 light:dark environment at regulated temperature. Three treatments were used: 1) cont: animals underwent no surgical procedure (n=6 animals); 2) axotomy: animals received bilateral axotomy of the SCG and survived for either 24 hours (n=5 animals) or 7 days (n=5 animals); 3) injury: animals received compression injury of the internal and external carotid nerves and survived for either 24 hours (n=7 animals) or 7 days (n=7 animals). All methods used in this study were approved by the

Miami University Institutional Animal Care and Use Committee and efforts were taken to minimize discomfort and pain to the animals.

Axotomy/injury procedures

Young adult female Sprague Dawley rats (200–225 gm; Harlan Labs) were anesthetized with intramuscular injection of Ketamine (80 mg/kg):Rompun (14 mg/kg) cocktail. A ventral incision approximately 3 cm in length was made in the neck region. The postganglionic nerves of the SCG were exposed and both the external carotid nerve (ECN) and internal carotid nerve (ICN) were gently separated from surrounding tissues. For bilateral axotomy, nerves were transected approximately 2 mm from their origin in the SCG with microdissecting scissors (Nagata et al., 1987; Sun and Zigmond 1996) and the proximal and distal ends of each nerve were then folded under. The procedure was repeated on the other side. For bilateral compression injury, the ICN and ECN each were crushed with flat forceps for approximately 3 seconds. In order to control for any imperfections in the forceps surface used for crushing, the forceps were turned over and the crush procedure was repeated for an additional 3 seconds. The incision was closed using sutures and tissue glue (Nexaband, Phx, AZ). After a 24 hour or 7 day survival, animals were sacrificed via decapitation using a Harvard guillotine apparatus. The SCG from the left and right sides was removed and snap frozen in liquid nitrogen and stored at -80°C until further processing.

In order to assess the extent of injury in the compression injury cases, the SCG's were probed for activating transcription factor-3 (ATF-3), an injury marker shown to be upregulated following axotomy (Hyatt Sachs et al., 2007) as well as other forms of neuronal damage (Peters et al., 2007a, 2007b). All injured cases used in the analyses showed an upregulation of ATF-3, indicating injury to the post-ganglionic axons.

Protein isolation and semi-quantitative western blot analysis

Protein from each SCG was obtained by sonicating tissues in 10mM Tris-HCl buffer (pH 7.4) containing 1% SDS and 1% protease inhibitor cocktail (Sigma-Aldrich). Total protein concentration was determined using a BCA assay (Pierce Biotechnology, Inc.) and samples were prepared as described by Laemmli (1970). For NGF and NT-3, lysate from individual SCG's (20 μg for NGF, 20 μg for NT-3) and 2.5S NGF (10 ng; Harlan Labs) or recombinant human NT-3 (10 ng; Chemicon) peptides were loaded and run on a 5% SDS-polyacrylamide gel electrophoresis (PAGE) stacking gel and 12% SDS-PAGE resolving gel. Lysate from at least 3–5 individual SCG's per treatment were loaded onto a single gel. Proteins were transferred onto PVDF membrane overnight at 4°C and at a constant 150 mAmps for 16 hours to total 2,300 mAmps in transfer buffer (25mM Tris, 192mM glycine, 10% (v/v) methanol). On the outer lanes of each SDS-PAGE gel, Precision Plus protein unstained standards (Bio-Rad Laboratories) were loaded for estimation of molecular weight. The membrane containing protein was incubated in 4% non-fat dry milk diluted in Tris Buffered Saline with Tween-20 (TBST) for 4 hr at room temperature, incubated overnight at 4°C in either rabbit anti-NGF (H-20, 1:1000; Santa Cruz Biotechnology; Lot #H1105), rabbit anti-NT-3 (N-20, 1:1000; Santa Cruz; Lot #C2505), or goat anti-NT-3 (1:500; Santa Cruz; Lot #K1203), rinsed with TBST, and incubated 2 hr at room temperature in goat anti-rabbit horseradish peroxidase (HRP) IgG (1:10,000 for NGF, 1:100,000 for rabbit NT-3; Chemicon) or donkey anti-goat HRP IgG (1:50,000 for goat NT-3; Santa Cruz). All membranes were rinsed with TBST, covered with SuperSignal West Pico Chemiluminescent Substrate (Pierce Biotechnology, Inc.) for 5 min, and then placed in an autoradiography cassette for development. Membrane containing protein was stripped and reprobed for glyceraldehyde 3-phosphate dehydrogenase (GAPDH; 1:80,000, mouse anti-GAPDH, 1:100,000, goat anti-mouse HRP IgG; Chemicon). For analysis of tyrosine hydroxylase (TH), SCG lysate (5 μg total protein) was loaded and run as described for NGF and NT-3 protein analyses. Membrane was incubated overnight at 4°C in mouse anti-TH

(1:200,000; BD Biosciences), rinsed with TBST, incubated 2 hours at room temperature in goat anti-mouse HRP IgG (1:80,000).

Antibody specificity was determined by omission of primary antibody from the western blot protocol. In addition, no signal was observed when membrane was incubated overnight at 4°C in solution containing rabbit NGF or rabbit NT-3 antibody with 10-fold excess of appropriate blocking peptide (Santa Cruz Biotechnology; Randolph et al., 2007). The same lot # of NGF antibody was used throughout the study. Similar staining patterns were found using the two different NT-3 antibodies described above.

Films were scanned into ImageQuant 5.2 (Amersham Biosciences) and densitometry readings for all bands were obtained with the local background subtracted. The densitometry readings for NGF, NT-3, or TH bands were normalized to GAPDH for each sample to produce a ratio. A mean of the ratios was obtained for the control and comparisons were then made to the ratios obtained from each of the axotomized tissues or injured tissues. A minimum of 3 SCG's per treatment were subjected to analysis using the Mann-Whitney test. Values are reported as mean $\pm$ SEM. Significance was reported at $p < 0.05$. The data obtained from the immunoblots shown in Figures 1, 3, and 4 were used to generate the graphs shown in Figures 2–4. In addition, at least 3 additional SCG's for each treatment were examined to confirm the findings obtained in the quantitative analyses.

Acknowledgements

This study was supported by NIH NS051206 awarded to LGI and the Miami University Summer Scholars Program at Miami University that supported RGW and AF. Thanks to Alec Lawrence for his assistance with the western blot procedures.

References

- Bierl MA, Isaacson LG. Increased pro-forms in aged sympathetic neurons and their targets. *Neurobiol Aging* 2007;28:122–134. [PubMed: 16377033]
- Bierl MA, Jones EE, Crutcher KA, Isaacson LG. 'Mature' nerve growth factor is a minor species in most peripheral tissues. *Neurosci Letters* 2005;380:133–137.
- Bussmann K, Sofroniew M. Re-expression of p75(NTR) by adult motor neurons after axotomy is triggered by retrograde transport of a positive signal from axons regrowing through damaged or denervated peripheral nerve tissue. *Neuroscience* 1999;91(1):273–281. [PubMed: 10336077]
- Dahlin LB, Archer DR, McLean WG. Axonal transport and morphological changes following nerve compression: an experimental study in the rabbit vagus nerve. *J Hand Surgery* 1993;18B:106–110.
- Dahlin LB, Sjostrand J, McLean WG. Graded inhibition of retrograde axonal transport by compression of rabbit vagus nerve. *J Neurol Sci* 1986;76:221–30. [PubMed: 2432189]
- Dahlin LB, Norborg C, McLean WG. Morphologic changes in nerve cell bodies induced by experimental graded nerve compression. *Exp Neurol* 1987;95:611–21. [PubMed: 3817083]
- Edwards RH, Selby MJ, Rutter WJ. Differential RNA splicing predicts two distinct nerve growth factor precursors. *Nature* 1986;319:784–787. [PubMed: 2419763]
- Fahnestock M, Michalski B, Xu B, Coughlin MD. The precursor pro-nerve growth factor is the predominant form of nerve growth factor in brain and is increased in Alzheimer's disease. *Mol Cell Neurosci* 2001;18:210–220. [PubMed: 11520181]
- Friedman W, Greene W. Neurotrophin signaling via trks and p75, *Exp. Cell Res* 1999;253:131–142.
- Hasan W, Pedchenko T, Krizsan-Agbas D, Baum L, Smith P. Sympathetic neurons synthesize and secrete pro-nerve growth factor protein. *J Neurobiol* 2003;57:38–53. [PubMed: 12973827]
- Hyatt Sachs H, Schreiber RC, Shoemaker SE, Sabe A, Reed E, Zigmond RE. Activating transcription factor 3 induction in sympathetic neurons after axotomy: response to decreased neurotrophin availability. *Neurosci* 2007;150:887–97.

- Korsching S, Thoenen H. Treatment with 6-hydroxydopamine and colchicine decreases nerve growth factor levels in sympathetic ganglia and increases them in the corresponding target tissues. *J Neurosci* 1985;5(4):1058–61. [PubMed: 2580066]
- Kwon K, Kim J, Chang B. Translocational changes of localization of synapsin in axonal sprouts of regenerating rat sciatic nerves after ligation crush injury. *J Vet Sci (Suwon-si, Korea)* 2000;1(1):1–9.
- Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680–685. [PubMed: 5432063]
- Nagata Y, Ando M, Takahama K, Iwata M, Hori S, Kato K. Retrograde transport of endogenous nerve growth factor in the superior cervical ganglion of adult rats. *J Neurochem* 1987;49:296–302. [PubMed: 3585337]
- Peters CM, Jimenez-Andrade JM, Jonas BM, Sevcik MA, Koewler NJ, Ghilardi JR, Wong GY, Mantyh PW. Intravenous paclitaxel administration in the rat induces a peripheral sensory neuropathy characterized by macrophage infiltration and injury to sensory neurons and their supporting cells. *Exp Neurol* 2007a;203:42–54. [PubMed: 17005179]
- Peters CM, Jimenez-Andrade JM, Kuskowski MA, Ghilardi JR, Mantyh PW. An evolving cellular pathology occurs in dorsal root ganglia, peripheral nerve and spinal cord following intravenous administration of paclitaxel in the rat. *Brain Res* 2007b;1168:46–59. [PubMed: 17698044]
- Peters EMJ, Hendrix S, Golz G, Klapp BF, Arck PC, Paus R. Nerve growth factor and its precursor differentially regulate hair cycle progression in mice. *J Histochem Cytochem* 2006;54:275–288. [PubMed: 16009967]
- Randolph CL, Bierl MA, Isaacson LG. Regulation of NGF and NT-3 protein expression in peripheral targets by sympathetic input. *Brain Research* 2007;1144:59–69. [PubMed: 17331480]
- Rattenholl A, Ruoppolo M, Flagiello A, Monti M, Vinci F, Marino G, Lilie H, Schwarz E, Rudolph R. Pro-sequence assisted folding and disulfide bond formation of human nerve growth factor. *J Mol Biol* 2001;305:523–533. [PubMed: 11152610]
- Reinshagen M, Geerling I, Eysselein VE, Adler G, Huff KR, Morre GP, Lakshmanan J. Commercial recombinant human β -nerve growth factor and adult rat dorsal root ganglia contain an identical molecular species of nerve growth factor prohormone. *J Neurochem* 2000;74:2127–2133. [PubMed: 10800958]
- Shoemaker SE, Kudwa AE, Isaacson LG. Sympathetic ingrowth to the trigeminal ganglion following intracerebroventricular infusion of nerve growth factor. *Brain Res* 2002;956:136–148. [PubMed: 12426056]
- Shoemaker SE, Hyatt Sachs H, Vaccariello SA, Zigmond RE. Reduction in nerve growth factor availability leads to a conditioning lesion-like effect in sympathetic neurons. *J Neurobiol* 2006;66:1322–1337. [PubMed: 16967509]
- Sun Y, Zigmond RE. Involvement of leukemia inhibitory factor in the increases in galanin and vasoactive intestinal peptide mRNA and the decreases in neuropeptide Y and tyrosine hydroxylase mRNA in sympathetic neurons after axotomy. *J Neurochem* 1996;67:1751–1760. [PubMed: 8858962]
- Thoenen H, Angeletti P, Levi-Montalcini R, Kettler R. Selective induction by nerve growth factor of tyrosine hydroxylase and dopamine-beta-hydroxylase in rat superior cervical ganglia (dopa-decarboxylase/monamine-oxidase). *PNAS* 1971;68 (7):1598–1602. [PubMed: 5283951]
- Yardley G, Relf B, Lakshmanan J, Reinshagen M, Moore GPM. Expression of nerve growth factor mRNA and its translation products in the anagen hair follicle. *Exp Dermatol* 2000;9:283–289. [PubMed: 10949551]
- Zhou XF, Chie ET, Deng YS, Rush RA. Rat mature sympathetic neurones derive neurotrophin 3 from peripheral effector tissues. *Eur J Neurosci* 1997;9(12):2753–64. [PubMed: 9517480]

NGF species in the SCG

Figure 1.

Western blot analysis of individual SCG's showed changes in NGF protein species in the SCG following axotomy or injury of the post-ganglionic nerves. The 22–24 kDa NGF species was decreased following axotomy (A., B.), yet was present and similar to control levels following injury (C., D.). The 73–75 kDa species was significantly increased following axotomy as well as injury after 24 hours (A.,C) but returned to normal levels after 7 days. NGF, NGF peptide (Harlan).

Figure 2.

Semi-quantitative analysis of NGF protein species following axotomy or injury revealed significant changes in specific NGF isoforms. The 22–24 kDa was significantly reduced following axotomy, but was similar to controls in the injured cases. The 73–75 kDa species was elevated at the 24 hr timepoint in both axotomy and injury treatments, but was relatively similar to control levels after 7 days. (20 µg loaded; n=3–4 different SCG's per treatment) (*p<0.05 compared with control)

Figure 3.

A. Western blot analysis of individual SCG's shows loss of mature NT-3 following axotomy, yet little change in this species was observed following injury. B. A significant decrease in the 'mature' 14 kDa isoform was observed following 24 hr and 7 day axotomy. However, no significant changes were observed in the injury cases (20 μ g loaded; n = 3–4 different SCG's per treatment) (* p <0.05 compared with control)

Figure 4. TH protein levels were dramatically reduced following axotomy, but only slightly reduced following injury. A. Western blot analysis of individual SCG's shows loss of TH following axotomy while a less dramatic decrease was observed in the injury cases. B. Semi-quantitative western blot analysis of TH protein in the SCG showed a dramatic 75% decrease following axotomy, with only a 25% decrease observed following injury (5 μ g loaded; n= 3–6 different SCG's per treatment) (*p < 0.05 compared with control).