Published in final edited form as:

Dev Neurobiol. 2009 May ; 69(6): 392–400. doi:10.1002/dneu.20706.

The Dependence on gp130 Cytokines of Axotomy Induced Neuropeptide Expression in Adult Sympathetic Neurons

Beth A. Habecker¹, Hilary Hyatt Sachs², Hermann Rohrer³, and Richard E. Zigmond^{2,*}

¹Department of Physiology and Pharmacology, Oregon Health Sciences University, Portland OR 97239-3098, USA

²Department of Neurosciences, Case Western Reserve University, Cleveland OH 44106-4975, USA

³Max-Planck Institute for Brain Research, 60528 Frankfurt, Germany

Abstract

Adult peripheral neurons exhibit dramatic changes in gene expression after axonal injury, including changes in neuropeptide phenotype. For example, sympathetic neurons in the superior cervical ganglion (SCG) begin to express vasoactive intestinal peptide (VIP), galanin, pituitary adenylate cyclase activating polypeptide (PACAP) and cholecystokinin after axotomy. Prior to these changes, nonneuronal cells in the SCG begin to express leukemia inhibitory factor (LIF). When the effects of axotomy were compared in LIF $-/-$ and wild type mice, the increases in VIP and galanin expression were less in the former, though significant increases still occurred. LIF belongs to a family of cytokines with overlapping physiological effects and multimeric receptors containing the subunit gp130. Real time PCR revealed large increases in the SCG after axotomy in mRNA for three members of this cytokine family, interleukin (IL)-6, IL-11, and LIF, with modest increases in oncostatin M, no changes in ciliary neurotrophic factor, and decreases in cardiotrophin-1. To explore the role of these cytokines, animals with selective elimination of the gp130 receptor in noradrenergic neurons were studied. No significant changes in mRNA levels for VIP, galanin, and PACAP were seen in ganglia from these mutant mice, while the increase in cholecystokinin was as large as that seen in wild type mice. The data indicate that the inductions of VIP, galanin and PACAP after axotomy are completely dependent on gp130 cytokines and that a second cytokine, in addition to LIF, is involved. The increase in cholecystokinin after axotomy, however, does not require the action of these cytokines.

Keywords

Cholecystokinin; Galanin; Pituitary adenylate cyclase activating polypeptide; Superior cervical ganglion; Vasoactive intestinal peptide

INTRODUCTION

Adult peripheral neurons exhibit dramatic changes in gene expression after axonal injury, among the largest of which are changes in neuropeptide expression (for review see Hökfelt et al., 1994; Zigmond et al., 1996). The first such observation was the decrease in substance P in sensory neurons in L4 and L5 dorsal root ganglia in response to sciatic nerve injury (Jessell et al., 1979). This decrease was subsequently shown to be accompanied by an

*Corresponding author: Richard E. Zigmond, Case Western Reserve University, School of Medicine, Department of Neurosciences, 10900 Euclid Avenue, Cleveland, OH 44106-4975, Telephone: 1-216-368-4614, Fax: 1-216-368-4650, E-mail address: rez@case.edu.

increase in another neuropeptide, vasoactive intestinal peptide (VIP; Nielsch & Keen, 1989; Noguchi et al., 1989).

Sympathetic neurons in the superior cervical ganglion (SCG) also exhibit changes in neuropeptide phenotype after axotomy. These include increases in expression of VIP (Hyatt Sachs et al., 1993), substance P (Rao et al., 1993a), galanin (Rao et al., 1993b), pituitary adenylate cyclase activating polypeptide (PACAP; Moller et al., 1997a) and cholecystokinin (Boeshore et al., 2004), together with decreases in neuropeptide Y (Sun & Zigmond, 1996). Changes similar to those that occur *in vivo* after axotomy also occur when SCG are maintained in explant culture (Zigmond et al., 1992; Rao et al., 1993a; Schreiber et al., 1994; Moller et al., 1997b).

Studies on neonatal sympathetic neurons in cell culture established that exogenous application of two cytokines, leukemia inhibitory factor (LIF) and ciliary neurotrophic factor (CNTF), stimulate changes in neuropeptide phenotype that are similar in some respects to those seen in adult neurons after axotomy or explantation, for example, increases in VIP and substance P (e.g., Ernsberger et al., 1989; Nawa et al., 1991; Freidin & Kessler, 1991). Subsequent experiments indicated that endogenous LIF plays a role in the changes in peptide expression that occur in the adult SCG after axotomy or explantation. Thus, *in vivo*, increases in VIP and galanin mRNA and peptide are significantly smaller after axotomy in LIF $-/-$ than in wild type mice (Rao et al., 1993b; Sun & Zigmond, 1996). In addition, the increase in VIP that occurs in explanted SCG is reduced when an antibody to LIF is added to the incubation medium (Sun et al., 1994). While barely detectable in the intact SCG, LIF mRNA increases dramatically in nonneuronal cells in the ganglion following axotomy or explantation (Banner & Patterson, 1994; Sun et al., 1994; Carlson et al., 1996; Sun & Zigmond, 1996; Sun et al., 1996).

LIF and CNTF belong to the gp130 family of cytokines. This family also includes cardiotrophin 1, cardiotrophin-like cytokine, interleukin (IL)-6, IL-11, neuropoietin/cardiotrophin-2, and oncostatin M. These cytokines differ considerably in amino acid sequence but are similar in secondary and tertiary structures and act on multimeric receptors all of which contain the signaling subunit gp130 (Taga, 1997). Given the facts that these cytokines produce overlapping physiological effects and that the peptide inductions after axotomy are reduced but not abolished in LIF $-/-$ mice, we decided to examine the possibility that other cytokines of this family are increased after axotomy. In addition, we examined peptide induction in animals in which the gp130 receptor subunit was selectively deleted in noradrenergic neurons.

MATERIALS AND METHODS

Wild type C57Bl/6 mice were purchased from The Jackson Laboratories (Bar Harbor, ME, USA) at six week old. Gp130 deficient mice (gp130^{DBHcre}) were generated by crossing gp130^{fl/fl} animals with animals having Cre recombinase expressed on the dopamine beta hydroxylase (DBH) promoter (Stanke et al., 2006). These strains had been previously back crossed to C57Bl/6 mice. Previous studies demonstrated that Cre-mediated recombination takes place in virtually all sympathetic neurons in the gp130^{DBHcre} mice (Stanke et al., 2006).

Mice were anesthetized by intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg), and neurons in one SCG were axotomized by unilateral transection of the major postganglionic nerves of the ganglion, the external and internal carotid nerves. Surgical procedures were approved by the Institutional Animal Care and Use Committee of Case Western Reserve University. Three experiments were performed. In the first, groups of five

wild type mice had their SCG unilaterally axotomized and both ganglia removed 6, 24, or 48 h later. Following this, cytokine mRNA levels were measured as described below. In the second experiment, four wild type and five gp130^{DBHcre} mice had their SCG unilaterally axotomized. Forty eight hours later both ganglia were removed and peptide mRNA levels were measured as described below. In the third experiment, five wild type and five gp130^{DBHcre} mice had their SCG unilaterally axotomized. Forty eight hours later, both ganglia were removed and immersion fixed for immunohistochemistry.

RNA isolation and real-time PCR

Axotomized and contralateral control ganglia were harvested at various times after surgery and stored immediately in RNAlater (Ambion, Austin, TX, USA). RNA was isolated from individual ganglia using the Ambion RNAqueous micro kit. Total RNA was quantified by OD260 using a NanoDrop spectrophotometer, and 200 ng of total RNA was reverse transcribed. Aliquots of a single reverse transcription reaction were used for all of the cytokine mRNA PCR reactions. Similarly, aliquots of a single reverse transcription reaction were used for all of the peptide mRNA PCR reactions. Real-time PCR was performed with the ABI TaqMan Fast Universal PCR Master mix in the ABI 7500, using exon-spanning ABI pre-validated TaqMan gene expression assays for cytokine and peptide genes. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was quantified as an internal control as it was found not to be altered by axotomy. This finding differs from that reported previously by Sun and Zigmond (1996) that GAPDH mRNA changes in the mouse SCG after axotomy, and we have no explanation for the different results. For the PCR amplification, 2 µl of RT reactions were used in a total volume of 20 µl, and each sample was assayed in duplicate. Cytokine and peptide mRNAs were normalized to GAPDH mRNA in the same sample.

Immunohistochemistry

SCG were removed from mice, desheathed, and fixed by immersion for 1–2 h in 4% paraformaldehyde. Ganglia were cryoprotected in graded sucrose (15% and 30%) and embedded in Tissue-Tek® O.C.T. compound (Fisher Scientific, Pittsburgh, PA, USA). Immunohistochemistry was performed on 10 µm cryostat sections. Rabbit antisera raised against phosphorylated signal transducer and activator of transcription (STAT) 3 (Tyr705; 1:100; Cell Signaling Technology, Danvers, MA, USA), galanin (1:1000; Millipore, Temecula, CA, USA), VIP (1:200; ImmunoStar, Hudson, WI, USA) and activating transcription factor (ATF) 3; (1:1000; sc-188; Santa Cruz Biotechnology Inc. Santa Cruz, CA, USA) were incubated with tissue sections at 4°C overnight. Sections were washed in three changes of phosphate buffered saline and incubated in a donkey anti-rabbit F(ab')₂ conjugated to Cy3 (1:400; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA).

To determine the percentage of cells containing ATF3-like immunoreactivity, sections were counterstained for 5 min with a solution of Hoechst 33258 (10 µg/ml; Sigma-Aldrich, St. Louis, MO, USA) and analyzed as described previously (Hyatt Sachs et al., 2007).

Statistics

Data are presented as means ± S.E.M. Differences in cytokine mRNA levels between surgical groups were analyzed by Student's t-test, two tailed, at each time point. Differences in neuropeptide and protein gene expression caused by surgery or genotype were analyzed by two-way ANOVA with the Bonferroni post-test.

RESULTS

Effects of axotomy on gp130 cytokines in the SCG

To obtain a profile of changes in expression of cytokines of the gp130 family after axotomy, IL-6, IL-11, LIF, oncostatin M, CNTF, and cardiotrophin 1 mRNA were measured in the ipsilateral and contralateral ganglion 6, 24, and 48 h after unilateral transection of the major postganglionic nerves of the mouse SCG. IL-6, IL-11, and LIF mRNA exhibited dramatic (approximately 20-fold or greater) increases at all three time points examined, except at 6 h for IL-11 (Fig. 1A–C). Oncostatin M mRNA showed a 3-fold increase at 6 h but no changes at later time points (Fig. 1D). Levels of CNTF mRNA were unaffected by axotomy (E), and cardiotrophin 1 mRNA decreased at 6 and 24 h after postganglionic nerve transection (Fig. 1E).

Phosphorylated STAT3 immunoreactivity in SCG after axotomy in wild type and gp130^{DBHcre} mice

Following the activation of their receptors, gp130 cytokines cause the phosphorylation of the enzyme Janus kinase and, subsequently, the phosphorylation and nuclear translocation of STAT3 (Taga, 1997). As controls for the blockade of gp130 cytokine action in the gp130^{DBHcre} mice, we examined sections of ganglia from sham-operated and axotomized animals for phospho-STAT3 immunoreactivity. For wild type mice, no labeled neurons were seen in ganglia from sham-operated animals, while many of the neurons were immunostained two days after axotomy (Fig. 2A). For gp130^{DBHcre} mice, no labeled neurons were seen in ganglia from either sham-operated or axotomized animals (Fig. 2B).

Changes in neuropeptide mRNA in SCG neurons after axotomy in wild type and gp130^{DBHcre} mice

To determine the role of gp130 cytokines in the axotomy induced increases in neuropeptide expression, experiments were performed in wild type and gp130^{DBHcre} mice. No differences in mRNA levels were found in sham-operated ganglia due to genotype. As expected based on previous studies in the rat (Hyatt Sachs et al., 1993; Mohny et al., 1994; Schreiber et al., 1994; Klimaschewski et al., 1994; Zhang et al., 1994; Moller et al., 1997a; Boeshore et al., 2004), 48 h after unilateral transection of the major postganglionic nerves of the SCG, VIP, galanin, PACAP, and cholecystokinin mRNA were increased in the ipsilateral SCG in wild type mice (Fig. 3A–D). The ratios of mRNA in the ipsilateral SCG to that in the contralateral ganglion for galanin, cholecystokinin, VIP, and PACAP were 7-, 8-, 16-, and 28-fold respectively. In marked contrast, when the ipsilateral SCG was compared to the contralateral ganglion after unilateral axotomy in gp130^{DBHcre} mice, no statistically significant differences in VIP, galanin, or PACAP mRNA were seen (Fig. 3A–C). For cholecystokinin mRNA, however, the increases in level after axotomy were not significantly different in wild type and gp130^{DBHcre} mice (Fig. 3D).

Changes in ATF3 mRNA and tyrosine hydroxylase (TH) after axotomy in wild type and gp130^{DBHcre} mice

The transcription factor ATF3 has been reported to increase after axotomy in sensory, motor, and sympathetic neurons (Tsujino et al., 2000; Hyatt Sachs et al., 2007). We previously reported, using immunohistochemistry, that the induction of ATF3 protein was equivalent in SCG from wild type and LIF knockout animals (Hyatt Sachs et al., 2007). To determine whether any of the cytokines of the gp130 family were involved in the regulation of ATF3, we examined ATF3 expression in gp130^{DBHcre} mice using both PCR and immunohistochemistry (see below). ATF3 mRNA increased dramatically in both wild type and gp130^{DBHcre} mice (45-fold, $p < 0.001$ and 37-fold, $p < 0.01$, respectively), though the

ratio of ATF3 mRNA to GAPDH mRNA was significantly higher for axotomized ganglia from the former genotype ($p < 0.001$; Fig. 3E).

In addition to increases in the expression of certain genes, axotomy leads to the decrease in expression of other genes (e.g., Boeshore et al., 2004). An example of the latter is the gene for TH (Koo et al., 1988; Sun and Zigmond, 1996). To determine whether the decrease in TH is dependent on gp130 cytokines, the effect of axotomy was examined in wild type and gp130^{DBHcre} mice. Axotomy of the SCG in wild type mice led to a 92% decrease in TH mRNA, while axotomy in gp130^{DBHcre} mice led to a significantly smaller, 63% decrease (Fig. 3F).

Immunoreactivity for neuropeptides and for ATF3 in SCG neurons after axotomy in wild type and gp130^{DBHcre} mice

Peptide immunoreactivity after axotomy was examined for VIP and galanin in wild type and gp130^{DBHcre} mice. Two days after axotomy, highly immunoreactive neurons were seen in wild type animals for both VIP (Fig. 4A) and galanin (Fig. 4C); however, no such cells were seen in the gp130^{DBHcre} mice (Fig. 4B, D).

We previously reported that the percentage of neurons in the SCG that expressed ATF3-like immunoreactivity was the same in ganglia from wild type and LIF knockout animals (Hyatt Sachs et al., 2007). We, therefore, examined ATF3 immunoreactivity in axotomized ganglia in wild type and gp130^{DBHcre} mice and found the same percentage of immunostained neurons for both genotypes (wild type: $54.4 \pm 2.8\%$ and gp130^{DBHcre}: $51.1 \pm 2.8\%$; Fig. 4E, F).

DISCUSSION

Axotomy produces large changes in gene expression in peripheral neurons. In a microarray study on the rat SCG 48 h after transection of the major postganglionic nerve trunks, the category of proteins whose mRNA expression was most dramatically increased was that of neuropeptides (Boeshore et al., 2004). Included among the genes whose expression increased were cholecystokinin, galanin, PACAP, and VIP. Data from real time PCR assays in the present study show that these peptides increased similarly after axotomy in the SCG of wild type mice (Fig. 3).

Thus far two signals have been identified as triggering changes in gene expression after axotomy, namely, an increase in the cytokine LIF and a decrease in the neurotrophin nerve growth factor (NGF). Though not detectable in the intact SCG, LIF mRNA is measurable in the rat ganglion within an hour after axotomy, reaching peak values at 6 h (Sun et al., 1996; Sun & Zigmond, 1996). The current study showed strikingly elevated LIF mRNA levels in mouse SCG 6, 24, and 48 h after axotomy. Two types of evidence support the role of LIF in the regulation of neuropeptide levels in sympathetic neurons after axotomy. First when the SCG from LIF^{-/-} mice are subjected to axotomy, the increases in VIP and galanin mRNA and peptide are substantially less than those seen in wild type animals, though significant increases still occur (Rao et al., 1993b; Sun & Zigmond, 1996). Second, addition of LIF to organ and cell cultures of SCG from LIF^{-/-} animals increases VIP and galanin expression, respectively (Rao et al., 1993b; Klimaschewski, 1997).

Because LIF belongs to a family of cytokines that have overlapping physiological effects, we sought to determine whether any of the other cytokines in the family increased in the mouse SCG after axotomy. In addition to LIF, we found large increases in IL-6 and IL-11 and a smaller increase in oncostatin M. To determine the effect of blockade of all cytokines in this family on axotomy induced peptide expression, we examined mice in which gp130

was knocked out in noradrenergic neurons, such as the neurons of the SCG. Evidence supporting the idea that cytokine action was blocked in these animals was the absence of STAT3 phosphorylation after axotomy. In addition, previous developmental studies with these animals established that the cytokine dependent cholinergic switch in the sympathetic innervation of the sweat gland is absent in these animals (Stanke et al., 2006). Strikingly, in these conditional knockout animals, no significant increases in VIP, galanin, or PACAP were seen after axotomy. Together with the partial effects on peptide induction previously seen in LIF knockout animals, these results suggest two conclusions: first, that one or more gp130 cytokines in addition to LIF is involved in regulating these peptides after axotomy, and second, that the induction of these neuropeptides is completely dependent on cytokine signaling.

Unlike the complete abolition of changes in VIP, galanin and PACAP expression in the gp130^{DBHcre} mice, these conditional knockout animals showed more subtle effects on the regulation of TH and ATF3 mRNA after axotomy. As previously reported for LIF ^{-/-} mice, the gp130^{DBHcre} mice exhibited a smaller decrease in TH mRNA after axotomy than that seen in wild type mice, suggesting that cytokine suppression of TH expression is partially responsible for the decrease in TH mRNA after axotomy. Similarly, the conditional knockout mice showed a smaller increase in ATF3 mRNA than did wild type animals. In spite of this difference in expression of ATF3 mRNA, the percentage of neurons in the SCG exhibiting ATF3-like immunoreactivity was the same in both genotypes. This apparent discrepancy between ATF3 mRNA and protein may reflect a difference in the translation of the mRNA or in the turnover of the protein in the two genotypes, but, alternatively, it may simply reflect a decrease in the amount of ATF3-like immunoreactivity per neuron, something we did not measure in this study.

In addition to inducing cytokines in the SCG, axotomy leads to a decrease in levels of NGF in the ganglion (Nagata et al., 1987; Shoemaker et al., 2006). The role of endogenous NGF in regulating neuropeptide expression has been studied by injecting intact rats with an antiserum raised against NGF. Animals treated in this way showed elevated concentrations of VIP and galanin mRNA and peptide in the SCG (Shadiack et al., 1998; 2001). On the other hand, application of exogenous NGF to the axotomized SCG inhibited the increases in these peptides normally seen after axotomy (Shadiack et al., 2001).

Given the finding that reduced NGF availability stimulates VIP and galanin expression in the rat SCG, it is noteworthy that no significant stimulation of VIP and galanin occurs after axotomy in gp130^{DBHcre} mice, since it would be expected that NGF levels would be decreased in the SCG in these animals. One possible explanation is that the effects on peptide expression of administering antiserum to NGF are mediated by cytokine induction. This remains a possibility, because although we found no increase in LIF expression in the rat SCG after antiserum treatment, other cytokines were not measured (Shadiack et al., 1998). Together the results suggest either that there is a species difference in the effectiveness of decreased neurotrophin availability in triggering peptide gene expression or that there is an interaction between the cytokine and neurotrophin mechanisms for regulating neuropeptide levels, whereby in the absence of cytokine signaling, lowering NGF levels loses its effectiveness in triggering peptide gene expression.

In striking contrast to the regulation of VIP, galanin, and PACAP, the increased expression of cholecystokinin after axotomy appears to be completely independent of the signaling by gp130 cytokines. Whether this peptide is sensitive to NGF withdrawal or to some yet unknown signal remains to be determined. Also to be established is whether the changes in cholecystokinin occur in a subpopulation of SCG neurons, different from those in which the other peptides are induced.

What are the functional consequences of these cytokine dependent and independent changes in neuropeptide expression in sympathetic neurons? It has long been hypothesized that the changes in gene expression that occur in peripheral neurons following axotomy underlie a shift in the neuron from a cell engaged in synaptic transmission to one engaged in regeneration (e.g., Grafstein, 1975). Experiments with knockout mice for specific neuropeptides have established roles for galanin and PACAP in regeneration of sensory and motor neurons, respectively (Holmes et al., 2000; Sachs et al., 2007; Armstrong et al., 2008). Whether these peptides play similar roles in regeneration of sympathetic neurons remains to be established.

Acknowledgments

Supported by NIH grants NS17512 to R.E.Z. and HL68231 to B.A.H. and grants from the DFG, Schram-Stiftung and Wilhelm Sander-Stiftung to H.R.

References

- Armstrong BD, Abad C, Chhith S, Cheung-Lau G, Hajji OE, Nobuta H, Waschek JA. Impaired nerve regeneration and enhanced neuroinflammatory response in mice lacking pituitary adenylyl cyclase activating peptide. *Neuroscience*. 2008; 151:63–73. [PubMed: 18055122]
- Banner LR, Patterson PH. Major changes in the expression of the mRNAs for cholinergic differentiation factor/leukemia inhibitory factor and its receptor after injury to adult peripheral nerves and ganglia. *Proc Natl Acad Sci USA*. 1994; 91:7109–7113. [PubMed: 8041754]
- Boeshore KL, Schreiber RC, Vaccariello SA, Sachs HH, Salazar R, Lee J, Ratan RR, Leahy P, Zigmond RE. Novel changes in gene expression following axotomy of a sympathetic ganglion: a microarray analysis. *J Neurobiol*. 2004; 59:216–235. [PubMed: 15085539]
- Carlson CD, Bai Y, Ding M, Jonakait GM, Hart RP. Interleukin-1 involvement in the induction of leukemia inhibitory factor mRNA expression following axotomy of sympathetic ganglia. *J Neuroimmunol*. 1996; 70:181–190. [PubMed: 8898726]
- Ernsberger U, Sendtner M, Rohrer H. Proliferation and differentiation of embryonic chick sympathetic neurons: effects of ciliary neurotrophic factor. *Neuron*. 1989; 2:1275–1284. [PubMed: 2576210]
- Freidin M, Kessler JA. Cytokine regulation of substance P expression in sympathetic neurons. *Proc Natl Acad Sci USA*. 1991; 88:3200–3203. [PubMed: 1707535]
- Grafstein B. The nerve cell body response to axotomy. *Exp Neurol*. 1975; 48:32–51. [PubMed: 1102323]
- Hökfelt T, Zhang X, Wiesenfeld-Hallin Z. Messenger plasticity in primary sensory neurons following axotomy and its functional implications. *Trends Neurosci*. 1994; 17:22–30. [PubMed: 7511846]
- Holmes FE, Mahoney S, King VR, Bacon A, Kerr NC, Pachnis V, Curtis R, Priestley JV, Wynick D. Targeted disruption of the galanin gene reduces the number of sensory neurons and their regenerative capacity. *Proc Natl Acad Sci USA*. 2000; 97:11563–11568. [PubMed: 11016970]
- Hyatt-Sachs H, Schreiber RC, Bennett TA, Zigmond RE. Phenotypic plasticity in adult sympathetic ganglia in vivo: effects of deafferentation and axotomy on the expression of vasoactive intestinal peptide. *J Neurosci*. 1993; 13:1642–1653. [PubMed: 8463841]
- 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. *Neuroscience*. 2007; 150:887–897. [PubMed: 18031939]
- Jessell T, Tsunoo A, Kanazawa I, Otsuka M. Substance P: depletion in the dorsal horn of rat spinal cord after section of the peripheral processes of primary sensory neurons. *Brain Res*. 1979; 168:247–259. [PubMed: 221070]
- Klimaschewski L. Regulation of galanin in rat sympathetic neurons in vitro. *Neurosci Lett*. 1997; 234:87–90. [PubMed: 9364504]
- Klimaschewski L, Tran TD, Nobiling R, Heym C. Plasticity of postganglionic sympathetic neurons in the rat superior cervical ganglion after axotomy. *Microsc Res Tech*. 1994; 29:120–130. [PubMed: 7529068]

- Koo EH, Hoffman PN, Price DL. Levels of neurotransmitter and cytoskeletal protein mRNAs during nerve regeneration in sympathetic ganglia. *Brain Res.* 1988; 449:361–363. [PubMed: 2899448]
- Mohney RP, Siegel RE, Zigmond RE. Galanin and vasoactive intestinal peptide messenger RNAs increase following axotomy of adult sympathetic neurons. *J Neurobiol.* 1994; 25:108–118. [PubMed: 7517435]
- Moller K, Reimer M, Ekblad E, Hannibal J, Fahrenkrug J, Kanje M, Sundler F. The effects of axotomy and preganglionic denervation on the expression of pituitary adenylate cyclase activating peptide (PACAP), galanin and PACAP type 1 receptors in the rat superior cervical ganglion. *Brain Res.* 1997a; 775:166–182. [PubMed: 9439840]
- Moller K, Reimer M, Hannibal J, Fahrenkrug J, Sundler F, Kanje M. Pituitary adenylate cyclase-activating peptide (PACAP) and PACAP type 1 receptor expression in regenerating adult mouse and rat superior cervical ganglia in vitro. *Brain Res.* 1997b; 775:156–165. [PubMed: 9439839]
- Nagata Y, Ando M, Takahama K, Iwata M, Hori S, Kato K. Retrograde transport of endogenous nerve growth factor in superior cervical ganglion of adult rats. *J Neurochem.* 1987; 49:296–302. [PubMed: 3585337]
- Nawa H, Nakanishi S, Patterson PH. Recombinant cholinergic differentiation factor (leukemia inhibitory factor) regulates sympathetic neuron phenotype by alterations in the size and amounts of neuropeptide mRNAs. *J Neurochem.* 1991; 56:2147–2150. [PubMed: 1902872]
- Nielsen U, Keen P. Reciprocal regulation of tachykinin- and vasoactive intestinal peptide-gene expression in rat sensory neurones following cut and crush injury. *Brain Res.* 1989; 481:25–30. [PubMed: 2706464]
- Noguchi K, Senba E, Morita Y, Sato M, Tohyama M. Prepro-VIP and preprotachykinin mRNAs in the rat dorsal root ganglion cells following peripheral axotomy. *Brain Res Mol Brain Res.* 1989; 6:327–330. [PubMed: 2593782]
- Rao MS, Sun Y, Vaidyanathan U, Landis SC, Zigmond RE. Regulation of substance P is similar to that of vasoactive intestinal peptide after axotomy or explantation of the rat superior cervical ganglion. *J Neurobiol.* 1993a; 24:571–580. [PubMed: 7686961]
- Rao MS, Sun Y, Escary JL, Perreau J, Tresser S, Patterson PH, Zigmond RE, Brulet P, Landis SC. Leukemia inhibitory factor mediates an injury response but not a target-directed developmental transmitter switch in sympathetic neurons. *Neuron.* 1993b; 11:1175–1185. [PubMed: 7506046]
- Sachs HH, Wynick D, Zigmond RE. Galanin plays a role in the conditioning lesion effect in sensory neurons. *Neuroreport.* 2007; 18:1729–1733. [PubMed: 17921877]
- Schreiber RC, Hyatt-Sachs H, Bennett TA, Zigmond RE. Galanin expression increases in adult rat sympathetic neurons after axotomy. *Neuroscience.* 1994; 60:17–27. [PubMed: 7519758]
- Shadiack AM, Sun Y, Zigmond RE. Nerve growth factor antiserum induces axotomy-like changes in neuropeptide expression in intact sympathetic and sensory neurons. *J Neurosci.* 2001; 21:363–371. [PubMed: 11160417]
- Shadiack AM, Vaccariello SA, Sun Y, Zigmond RE. Nerve growth factor inhibits sympathetic neurons' response to an injury cytokine. *Proc Natl Acad Sci USA.* 1998; 95:7727–7730. [PubMed: 9636218]
- Shoemaker SE, Sachs HH, 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]
- Stanke M, Duong CV, Pape M, Geissen M, Burbach G, Deller T, Gascan H, Otto C, Parlato R, Schutz G, Rohrer H. Target-dependent specification of the neurotransmitter phenotype: cholinergic differentiation of sympathetic neurons is mediated in vivo by gp 130 signaling. *Development.* 2006; 133:141–150. [PubMed: 16319110]
- 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]
- Sun Y, Rao MS, Zigmond RE, Landis SC. Regulation of vasoactive intestinal peptide expression in sympathetic neurons in culture and after axotomy: the role of cholinergic differentiation factor/leukemia inhibitory factor. *J Neurobiol.* 1994; 25:415–430. [PubMed: 8077967]

- Sun Y, Landis SC, Zigmond RE. Signals triggering the induction of leukemia inhibitory factor in sympathetic superior cervical ganglia and their nerve trunks after axonal injury. *Mol Cell Neurosci.* 1996; 7:152–163. [PubMed: 8731483]
- Taga T. The signal transducer gp130 is shared by interleukin-6 family of haematopoietic and neurotrophic cytokines. *Ann Med.* 1997; 29:63–72. [PubMed: 9073325]
- Tsujino H, Kondo E, Fukuoka T, Dai Y, Tokunaga A, Miki K, Yonenobu K, Ochi T, Noguchi K. Activating transcription factor 3 (ATF3) induction by axotomy in sensory and motoneurons: A novel neuronal marker of nerve injury. *Mol Cell Neurosci.* 2000; 15:170–182. [PubMed: 10673325]
- Zhang X, Dagerlind A, Bao L, Ji RR, Lundberg JM, Hökfelt T. Increased expression of galanin in the rat superior cervical ganglion after pre- and postganglionic nerve lesions. *Exp Neurol.* 1994; 127:9–22. [PubMed: 7515354]
- Zigmond RE, Hyatt Sachs H, Baldwin C, Qu XM, Sun Y, McKeon TW, Schreiber RC, Vaidyanathan U. Phenotypic plasticity in adult sympathetic neurons: changes in neuropeptide expression in organ culture. *Proc Natl Acad Sci USA.* 1992; 89:1507–1511. [PubMed: 1741405]
- Zigmond RE, Hyatt Sachs H, Mohney RP, Schreiber RC, Shadiack AM, Sun Y, Vaccariello SA. Changes in neuropeptide phenotype after axotomy of adult peripheral neurons and the role of leukemia inhibitory factor. *Perspect Dev Neurobiol.* 1996; 4:75–90. [PubMed: 9169921]

Fig. 1.

Changes in expression of mRNA for gp130 cytokines in the SCG after axotomy. Six, twenty four and forty eight hours after unilateral axotomy of neurons in the SCG, ipsilateral (Ax) and contralateral (Sh) ganglia were examined by real time PCR for their levels of mRNA for IL-6, IL-11, LIF, oncostatin M (OSM), CNTF, and cardiotrophin 1 (CT1). Data in this figure and in all other histograms are presented as means $\pm$ S.E.M. mRNAs for IL-6 (A), IL-11 (B), and LIF (C) were significantly increased in the ipsilateral ganglia at all time points examined, except at 6 h for IL-11. OSM mRNA was significantly elevated in the ipsilateral ganglion at 6 h after axotomy but not at later times examined (D). CNTF mRNA was unchanged at all three time points (E), while CT1 mRNA was significantly lower in the ipsilateral ganglion at 6 h and 24 h after axotomy (F). * indicates $p < 0.05$ compared to sham-operated ganglia at the same time point.

Fig. 2. Phospho-STAT3 immunostaining in sections of SCG after axotomy. Forty eight hours after unilateral axotomy, phospho-STAT3 immunostaining was seen in sections of the ipsilateral SCG from wild type (A), but not from gp130^{DBHcre} (B), mice. Scale bar = 50 μ m.

Fig. 3.

Regulation of gene expression in the SCG after axotomy in gp130 conditional knockout mice. Wild type and gp130^{DBHcre} mice were subjected to unilateral axotomy of the SCG, and mRNA levels for several neuropeptides and proteins were measured 48 h later in the ipsilateral (Ax) and contralateral (Sh) ganglia. Data are presented as means $\pm$ S.E.M. mRNA for VIP (A), galanin (B), and PACAP (C) increased significantly in the ipsilateral ganglia of the wild type mice, but not in those of the knockout animals. Cholecystokinin (CCK) mRNA was increased similarly in axotomized ganglia of animals of both genotypes (D). ATF3 mRNA increased (E) and TH mRNA decreased (F) in ganglia of animals of both genotypes, although the magnitude of the changes were significantly larger in wild type than in

knockout animals. * indicates $p < 0.05$ compared to sham-operated ganglia of the same genotype.

Fig. 4. VIP-, galanin-, and ATF3-like immunoreactivity in axotomized SCG from wild type and gp130^{DBHcre} mice. Forty eight hours after unilateral axotomy, VIP- and galanin-like immunoreactivity was present in axotomized ganglia from wild type mice (A,C) but not in similarly treated ganglia from gp130^{DBHcre} mice (B,D). In contrast ATF3-like immunoreactivity was found in axotomized ganglia from both wild type (E) and gp130^{DBHcre} (F) mice. Scale bar = 50 μ m.