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Perivascular Cells Increase Expression of Ciliary Neurotrophic Factor Following Partial Denervation of the Rat Neurohypophysis

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Abstract

The expression of ciliary neurotrophic factor (CNTF) was investigated immunocytochemically during the axonal degeneration and collateral axonal sprouting response that follows partial denervation of the rat neurohypophysis. A significant increase in the number of CNTF-immunoreactive (CNTF-ir) cells was observed in the neurohypophysis of partially denervated animals compared to age-matched sham-operated controls by 5 days post-denervation, remaining elevated throughout the 30 day post denervation period. Stereometric assessment of the numbers of CNTF-ir cells within the partially denervated neurohypophysis demonstrated a 36% increase by 3 days following denervation reaching 130% of control values by 10 days post-lesion. The cell numbers remained elevated throughout the 30 days post-lesion period suggesting that CNTF may play a role in the neurosecretory axonal sprouting process known to occur between 10 and 30 days post-denervation. Subsequent preparations pairing anti-CNTF with antibodies against ED1, CR3, p75 low affinity neurotrophin receptor (p75^{LNGFR}), and S100 β , demonstrated that CNTF was exclusively localized in a phenotypically-distinct population of perivascular cells. The association of perivascular cells with phagocytic activity was confirmed by dual label fluorescence microscopy showing the colocalization of P75^{LNGFR}-ir and OX-42-ir in cells expressing the ED-1 antigen. No increase in CNTF-ir was observed in non-injured animals in which heightened levels of neurosecretory activity were induced physiologically. These results suggest that increased CNTF-ir occurs in response to conditions which induce high levels of phagocytic activity by perivascular cells in the axotomized neurohypophysis which is sustained throughout a period in which axonal sprouting is known to occur in the partially denervated neurohypophysis.

Keywords

CNTF; Perivascular Cell; Axotomy; Magnocellular Neurosecretory System; Neurohypophysis; Immunocytochemistry

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Introduction

The magnocellular neurosecretory system (MNS) is comprised of the neurons which form the hypothalamic supraoptic (SON) and paraventricular (PVN) nuclei. Their respective axons project to the neurohypophysis of the pituitary via the hypothalamo-neurohypophysial tract where they terminate on the basement membrane of the capillary plexus (Silverman, 1983). Within the neurohypophysis these unmyelinated axons and terminals are closely associated with pituicytes, a specialized form of astrocyte (Salm et al., 1982) parenchymal microglia (Pérez-Figueré et al., 1986; Pow et al., 1989) and perivascular cells (Olivieri-Sangiaco, 1972). Unique among CNS systems, the MNS is characterized by a high propensity for axon terminal reorganization following injury (Billenstein and Leveque, 1955; Moll, 1957; Adams et al., 1969; Becket et al., 1969; Raisman, 1973; Antunes et al., 1980; Silverman and Zimmerman, 1982). This well documented regenerative capability led us to investigate the ability of MNS axons to undergo collateral sprouting following partial denervation of the neurohypophysis (Watt et al., 1991; Watt and Paden, 1999) as well as the glial activities potentially involved in facilitating the axonal outgrowth (Watt and Paden, 2001 Watt et al., 2006). These studies show that unilateral transection of the hypothalamo-neurohypophysial tract results in an initial period of axonal degeneration followed by the restoration of the neurosecretory axon density within the neurohypophysis to normal values by 30 days post-lesion.

Although the specific factors that promote the terminal sprouting response remain ill defined, there is evidence that ciliary neurotrophic factor (CNTF) plays an important role. We have demonstrated the endogenous expression of CNTF in identified astrocytes within the SON, which is elevated in both the axotomized and the intact contralateral sprouting SON following unilateral lesion of the hypothalamo-neurohypophysial tract (Watt, et al., 2006). Others demonstrated that recombinant CNTF promotes survival of magnocellular neurons in organotypic cultures of PVN and SON nuclei (Vutskits et al., 1998; Rusnak et al., 2002). Furthermore, when an intact neurohypophysis was placed in the proximity to cultured PVN explants, neurosecretory process outgrowth toward the neurohypophysis was observed (Vutskits et al., 1998). Using RT-PCR analysis these authors also demonstrated the presence of CNTF message in the neurohypophysis and we have extended these observations to demonstrate the presence of CNTF-immunoreactive cells in the rat neurohypophysis (Watt et al., 2006). These data have led us to hypothesize that CNTF acts as a target derived trophic factor within the magnocellular axonal terminal field, supporting the collateral sprouting of vasopressinergic axons. In order to further explore the role of CNTF in neurosecretory plasticity, we have defined phenotypically the cellular source of CNTF in the neurohypophysis and have quantified the temporal changes in cellular expression of CNTF which accompanies the axonal degeneration and sprouting response.

Methods

Animals

All animals included in this study were male Sprague-Dawley rats of approximately 150–250 gms body weight at the time a unilateral hypothalamic knife cut of the hypothalamo-neurohypophysial tract was performed. Sham surgical controls were prepared under identical conditions except the knife cut did not penetrate the hypothalamus. All experimental animals were housed in the University of North Dakota Biomedical Research Facility, an AAALAC accredited facility, under a 12L:12D light cycle and had *ad lib* access to lab chow and tap water throughout the investigations. Experimental protocols utilized in these studies followed the guidelines outlined in the NIH Guide for the Care and Use of Laboratory Animals and were approved by the UND Institutional Animal Care and Use Committee.

Unilateral Lesion

Animals were anesthetized with a mixture of 42% ketamine, 42% xylazine, and 16% acepromazine administered at a dose of 0.75ml/kg followed by placement in a stereotaxic apparatus (Stoelting). An incision was made on the scalp to expose the skull, and the lesion coordinates marked with Bregma as stereotaxic zero. Lesion coordinates were; anterior/posterior (AP) -2 to +5mm and medial/lateral (ML) -0.5mm. A dremel drill was used to remove the skull along the lesion tract, after which, a wire knife was lowered, at AP -2mm, until it flexed as an indication of touching the ventral surface of the cranial cavity. The knife was brought up (dorsally) 0.5mm and brought forward to +5 AP to complete the lesion. This procedure results in the complete unilateral transection of the hypothalamo-neurohypophyseal tract, leading to a 42% reduction in neurosecretory input to the neurohypophysis (Watt and Paden, 1991). Following surgery, animals were individually housed until sacrificed at 1, 3, 5, 7, 10, and 30 days post-denervation. Complete unilateral transection of the hypothalamo-neurohypophyseal tract by the knife cut was verified histologically in every animal included in the studies described below. The effectiveness of the lesion preparation has been previously described in detail (Watt and Paden, 1991; Paden et al., 1995; Watt et al., 1999).

Tissue Preparation

All animals were perfused intracardially with cold saline for two minutes under deep isoflurane (Halocarbon Laboratories) anesthesia and then perfused for 20 minutes with a modified Nakane's periodate-lysine-paraformaldehyde (PLP) fixative prepared immediately before use (Paden et al., 1994). For light microscopic peroxidase immunocytochemistry the brain and neurohypophysis were removed intact and postfixed overnight in PLP, cryoprotected in 20% sucrose/PBS for 24–48 hours at 4°C and then snap frozen in OCT freezing compound (Ted Pella) in isopentane chilled in liquid nitrogen. Serial cryosections were then collected through the SON, PVN and NL and thaw mounted on gelatin-coated slides.

Immunocytochemistry

All sections used for peroxidase immunocytochemistry were first pretreated with 0.3% H₂O₂ in phosphate-buffered saline pH 7.4 (PBS) for 30 minutes followed by 4% of the appropriate normal sera (Vector) in PBS (blocking buffer) to reduce endogenous peroxidase activity and non-specific staining, respectively. All sections were washed repeatedly in PBS both before processing and between incubations.

For localization of anti-CNTF-immunoreactivity (CNTF-ir) sections were incubated sequentially in blocking buffer consisting of PBS with 4% normal horse serum (PBS/NHS; 1 hr at room temperature), goat anti-rat CNTF (1:100 in blocking buffer, R&D Systems), biotinylated horse anti-goat IgG (1:500 in PBS/NHS, 1 hr; Vector) and avidin-biotin complex in PBS for 1 hr (Vector ABC *Elite* kit). Binding of the ABC reagent was visualized using diaminobenzidine (Sigma) as chromogen with the generation of H₂O₂ by the glucose oxidase method (Itoh, et al., 1979). To further assess specificity of the polyclonal anti-CNTF antibody additional tissue sections were processed as described above with either omission of the primary antibody or using primary antibody that had been preabsorbed for 24 hours with a 10 fold molar excess of purified rat recombinant CNTF (R & D Systems).

For dual-peroxidase labeling pairing mouse anti-ED-1 (Serotec, 1:100) with mouse anti-OX42 (Serotec, 1:1000), mouse anti p75^{LNGFR} (1:5000, R&D Systems), rabbit anti-cow S100 β (1:5000; Accurate) or goat anti-CNTF (1:100) all incubation steps were performed at 4°C and separated by a minimum of three 10 minute PBS washes. Sections were treated as described above except ED-1-ir was visualized using .05% DAB containing 1% nickel-

chloride (Sigma) in the presence of 0.003% glucose oxidase (1mg/ml). The sections were then incubated overnight in the appropriate blocking buffer containing either mouse anti-OX42, mouse anti p75^{LNGFR} (1:5000, R&D Systems) rabbit anti-cow S100 β (1:5000; Accurate) or goat anti-CNTF. Following a 1 hr incubate in the appropriate biotinylated secondary antibodies and avidin-biotin complex (Vector) these primaries were visualized using DAB in the presence of glucose oxidase as described above.

For dual-label fluorescence labeling pairing goat anti-rat CNTF with rabbit anti-cow S100 β (1:5000; Accurate) or mouse anti p75^{LNGFR} (1:5000, R&D Systems) all incubation steps were performed at 4°C and separated by a minimum of three 10 minute PBS washes. Sections were initially treated with a PBS blocking buffer containing 4% normal serum of the appropriate species with 1% bovine serum albumin (Sigma) in PBS containing 0.1% Triton X-100 for 1 hr to reduce nonspecific staining. The sections were then incubated overnight in a solution of anti-S100 β or anti-p75^{LNGFR} in blocking buffer. Sections were then incubated sequentially in biotinylated horse anti-rabbit IgG (1:500; Jackson ImmunoResearch) and streptavidin-Cy3 (1:1000 in PBS, Jackson ImmunoResearch). The sections were then washed and incubated sequentially in blocking serum composed of 4% normal horse serum/PBS for 1 hr at 4°C, goat anti-rat CNTF overnight at 4°C followed with rabbit anti-goat conjugated to fluorescein isothiocyanate (FITC) (1:500, Jackson ImmunoResearch) for 1 hr at 4°C. The sections were then coverslipped with Vectashield (Vector), and images captured using an Olympus BX-51 fluorescent microscope with attached DP-71 color camera. Images were prepared for page-reproduction using Adobe Photoshop (v.6.0).

Quantitative Assessment of CNTF-ir Cell Numbers in the Neurohypophysis

The number of CNTF-ir cells/mm² in the neurohypophysis was determined on a minimum of 8 peroxidase-stained coronal sections from each lesion and littermate sham surgery-control animals sacrificed at post-surgery days PL1/Sh1, PL5, PL7, PL10 and PL30/Sh30 (PL=Post Lesion, Sh = Sham control, n=5 to 10 animals per group). Tissue sections were selected from approximately the same depths through the rostral to caudal axis of the neurohypophysis in all animals. Following peroxidase immunolabeling, the sections were counterstained with cresyl violet acetate (Sigma) to facilitate identification of individual cells. The CNTF-ir cells were then counted within the confines of the neurohypophysis using an Olympus BX-51 microscope with drawing tube attachment. The cross sectional area of each neurohypophysis section in which the CNTF-ir cells were counted was then measured using an MCID image analysis system (M4, Imaging Research). A single mean value representing the number of CNTF-ir cell/mm² was then calculated for each animal. These values were then used in an analysis of group differences through application of a univariate analysis of variance between groups and subsequent post-hoc comparisons using the least significance difference tests (SPSS v. 9.0).

Results

Immunophenotypic identification of CNTF-ir cells

CNTF-ir was investigated immunocytochemically between 1 and 30 days following unilateral lesion of the hypothalmo-neurohypophysial tract, and compared to the pattern of immunoreactivity in identically prepared age-matched littermate sham surgery controls. In the neurohypophysis of sham lesioned animals and intact controls, CNTF-ir was rarely observed, although occasional immunoreactive profiles could be found in the meningeal region in both groups. However, lesioned animals were characterized by a distinct increase in the number and intensity of CNTF-ir cells. These cells were characterized by a reticular appearance with elongated fine processes which were often found in close association with

blood vessels (Fig. 1A). In order to define the phenotype of the CNTF-ir cells in the neurohypophysis we first paired anti-CNTF with anti-S100 β , an astrocyte-specific Ca⁺⁺-binding protein (Cocchia and Miana, 1980). In contrast to our previous observations in the hypothalamus (Watt et al., 2006), in the neurohypophysis, no colocalization of S100 β -ir (yellow asterisks indicate green, FITC-labeled pituicytes) and anti-CNTF-ir (arrows, red, Cy3) was evident (Fig 1B). We next applied a well characterized microglial marker, monoclonal antibody OX-42 which recognizes the C3bi complement receptor. OX-42 is a pan macrophage marker and labels both parenchymal and perivascular microglial cells in the rat neurohypophysis (Pow et al., 1989). Subsequent examination of dual-peroxidase labeled sections revealed no co-localization when binding of OX-42 (purple, arrow) was localized simultaneously with anti-CNTF (brown, DAB)(Fig. 1C).

We have described previously a population of perivascular cells within the neurohypophysis which, at the light and ultrastructural level, are morphologically identical to OX-42-ir perivascular microglial cells, but are not themselves immunoreactive for the OX-42 antigen (Watt and Paden, 2001). However, these perivascular cells are immunoreactive for the p75 low affinity neurotrophin receptor (p75^{LNGFR}) (Yan et al., 1990; Watt and Paden, 2001). Therefore, we next paired anti-CNTF with anti-p75^{LNGFR} using dual fluorescence analysis. We found that all of the CNTF-ir cells identified within the neurohypophysis (Fig. 1D) were also immunoreactive for p75^{LNGFR} (Fig. 1E–F), although not all cells immunoreactive for the p75^{LNGFR} receptor were immunoreactive for CNTF (Fig. 1F).

To determine if the increase in CNTF-ir occurs in response to phagocytosis of degenerating neurosecretory axons, we used dual peroxidase immunolabeling to pair the anti-CNTF, OX-42, S100 β and P75^{LNGFR} antibody with an antibody that recognizes the ED-1 antigen. ED-1 is expressed on the surface of lysosomal membranes in CNS microglial cells and perivascular cells and is upregulated in the neurohypophysis following unilateral lesion (Watt and Paden, 2001). Similar to our previous observations (Watt and Paden, 2001), by 7 days post-denervation, extensive ED-1-ir is evident in the cytoplasm of OX-42-ir cells (Fig. 2A), and P75^{LNGFR}-ir (Fig. 2B) throughout the neurohypophysis. Although ED-1-ir was also observed occasionally in CNTF-ir cells (Fig. 2C), we found little conclusive evidence linking increased ED-1-ir with the occurrence of CNTF-ir. No indication of ED-1-ir was observed in S100 β -ir pituicytes indicating that they are not involved in the clearance of degenerating axons under these conditions (Fig. 2D).

CNTF-ir cells increase in the neurohypophysis following partial denervation

Between 3 and 30 days following unilateral lesion of the hypothalamo-neurohypophysial tract, an increase in the number of CNTF-ir cellular profiles was apparent when compared to age matched intact controls. Therefore, a quantitative assessment of the number of individual CNTF-ir cells was determined at 1, 3, 5, 7, 10 and 30 days post-denervation and age-matched littermate sham controls corresponding to lesion days 1 and 30. The cross sectional area of each section in which the CNTF-ir cells were counted was then measured using an MCID image analysis system (M4, Imaging Research). As demonstrated in Figure 3, this analysis confirmed that a significant increase in the number of CNTF-ir perivascular cells occurred following partial denervation (1 way ANOVA, $F=2.775$, $df=7, 49$, $P<.018$). The increase in the number of CNTF-ir cells in lesioned animals compared to 1 day or 30 day sham-operated controls was first significant at 7 days post-denervation (LSD test; $P<.04$) and reached a peak at 10 days post-denervation ($P<.003$). By 30 days following surgery the mean number of CNTF-ir cells remained elevated but had become much more variable between animals and no longer reached statistical significance.

Discussion

The magnocellular neurosecretory system provides an excellent model for investigating the role of neurotrophic support of compensatory axonal sprouting by CNS neurons. Following unilateral lesion of the hypothalamo-neurohypophyseal tract within the hypothalamus, a robust axonal sprouting response occurs within the partially denervated neurohypophysis which results in near complete recovery of axonal density by 30 days following the lesion (Watt and Paden, 1991; Watt et al., 1999). The sprouting response occurs at a distance from the lesion site and is not affected directly by the neural trauma. This allows examination of the processes occurring at the site of axonal sprouting in the absence of confounding inflammatory activity associated with hemorrhage and disruption of the blood brain barrier. In a previous study, we showed that CNTF is expressed endogenously in astrocytes within the SON and is upregulated following unilateral lesion within the lesioned and sprouting SON as well as the neurohypophysis. However, the CNTF-ir cell type within the neurohypophysis was not identified and its role in neurosecretory collateral sprouting was not addressed. Ciliary neurotrophic factor is known to play an important role as a neuronal survival factor (Sendtner et al., 1990; Magal et al., 1993; Larkfors et al., 1994; Burnham et al., 1994) and sprouting factor across a wide spectrum of CNS cell types (Siegel et al., 2000) including the magnocellular neurosecretory system (Vutskits et al., 1998; Rusnak et al., 2002, 2003). Indeed, Vutskits, et al., 1998 have shown that when explants of the PVN were cultured in the presence of neurohypophyseal explants process outgrowth originating from the PVN explants was observed to extend toward the neurohypophysis. These authors demonstrated further the presence of CNTF message in the neurohypophyseal tissue but again, the cellular source for CNTF was not identified within the neurohypophysis.

Here we report that the expression of CNTF is restricted to a distinct population of perivascular cells. CNTF-ir was invariably localized to cells that were p75^{LNGFR} positive/OX42 negative but never within perivascular microglial cells that were OX-42 positive/P75^{LNGFR} negative. This was observed regardless of the fact that both groups of cells are active phagocytes as evidenced by the increase the number of ED-1 profiles following axotomy. The initial increase in the number of CNTF-ir cells is temporally correlated with the onset of degeneration of severed axons and the subsequent phagocytic removal of the resulting axonal debris that occurs following partial deafferentation (Watt and Paden, 1991). In contrast, we have never observed changes in CNTF-ir or OX-42-ir following a variety of treatments that produce alterations in neurosecretory and/or pituitary activity in the neurohypophysis in the absence of injury, including dehydration and lactation. We observed no changes in the numbers of CNTF-ir cells or any alteration in the intensity of CNTF-ir in sham-lesioned animals. These observations are consistent with our previous ultrastructural studies demonstrating that P75^{LNGFR}-ir perivascular cells are phagocytically active following partial denervation of the neurohypophysis (Watt and Paden, 2001) and following bilateral electrolytic destruction of the paraventricular nuclei (Zambrano and De Robertis, 1968). However, given the consistent co-localization of CNTF-ir with P75^{LNGFR}-ir we were puzzled by our inability to routinely or conclusively co-localize ED-1-ir with CNTF-ir to the extent seen in P75^{LNGFR}-ir cells. One possible explanation may be that the more extensive labeling of cellular processes by the P75^{LNGFR} antibody would more readily capture the presence of ED-1 vesicles within the cell. Alternatively, the increased presence of CNTF-ir may be induced by as yet unidentified factors unrelated to phagocytic activity. Studies are currently underway pairing anterograde tracing with anti OX-42 and anti P75^{LNGFR} cellular markers to more closely examine induction of phagocytic/neuronophagic activity with the increased incidence of CNTF-ir.

What role the perivascular cells and their expression of CNTF may play in the initiation or maintenance of the sprouting response remains to be determined. It has been shown,

however, that impaired phagocytosis of degenerating axons is associated with a delayed onset of axonal sprouting in the denervated hippocampus (Steward 1992; Hoff et al., 1993). Microglial cells are known to produce neurotrophic substances in response to phagocytic activity. Indeed, *in vitro* studies have demonstrated that microglia can express nerve growth factor and NT-3 (Elkabes et al., 1996), release NGF (Mallet et al., 1989) and promote neuronal survival *in vitro* (Nagata et al., 1993).

Although the potential for release of CNTF from an intact cell remains controversial, recent studies support the concept that release of CNTF from non-injured astrocytes can occur, and show that cytokines, principally IL-1 β , may stimulate secretion (Kamiguchi et al., 1995; Reiness et al., 2001). We, and others, have demonstrated IL-1 β immunoreactivity and message in magnocellular neurons and its release from the dendrites and terminals of magnocellular SON neurons in response to osmotic stimulation (Watt and Hobbs, 1999; Widitz et al., 2003). Furthermore, the message for CNTFR α is expressed within the magnocellular divisions of the PVN and throughout the SON (MacLennan et al., 1996; Lee et al., 1997). Retrograde transport of the CNTF/CNTFR α complex has been shown to occur by sensory neurons, the rate of transport is increased in response to axotomy (Curtis et al., 1993), leading to increased neuronal survival (Sendtner et al., 1990; Siegel et al., 2000; Gurney et al., 2004).

In conclusion, we present evidence that an immunophenotypically-distinct subpopulation of perivascular cells increase their expression of CNTF, a neurotrophin which has been shown to promote sprouting from magnocellular neurons, following the induction of phagocytic activity. Our data show that the increase in CNTF-ir is sustained throughout the period in which axonal sprouting is known to occur, then begins to subside by 30 days post-denervation, a time by which the sprouting response is nearly complete (Watt and Paden, 1991). These data suggest that perivascular cells may contribute to the post-denervation regenerative response of magnocellular neurons.

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Reference List

- Adams JH, Daniel PM, Prichard MMI. Degeneration and regeneration of hypothalamic nerve fibers in the neurohypophysis after pituitary stalk section in the ferret. *J Comp Neurol* 1969;135:121–144. [PubMed: 5780526]
- Antunes JL, Louis KM, Huang S, Zimmerman E. Section of the pituitary stalk in the rhesus monkey: Morphological and endocrine observations. *Ann Neurol* 1980;8:308–316. [PubMed: 7002018]
- Beck E, Daniel PM, Prichard MM. Regeneration of hypothalamic nerve fibers in the goat. *Neuroendo* 1969;5:161–182.
- Billenstein DC, Leveque TF. The reorganization of the neurohypophysial stalk following hypophysectomy in the rat. *Endocrinology* 1955;56:704–717. [PubMed: 14391260]
- Cocchia D, Miana N. Immunocytochemical localization of the brain-specific S-100 protein in the pituitary gland of adult rat. *J of Neurocytol* 1980;9(6):771–782. [PubMed: 7009796]
- Curtis R, Adryan KM, Zhu Y, Harkness PJ, Lindsay RM, DiStefano PS. Retrograde axonal transport of ciliary neurotrophic factor is increased by peripheral nerve injury. *Nature* 1993 Sept 16;365(6443):253–255. [PubMed: 8371780]
- Elkabes S, DiCicco-Bloom EM, Black IB. Brain microglia/macrophages express neurotrophins that selectively regulate microglial proliferation and function. *J Neurosci* 1996;16(8):2508–2521. [PubMed: 8786427]

- Gurney ME, Yamamoto H, Kwon Y. Induction of motor neuron sprouting in vivo by ciliary neurotrophic factor and basic fibroblast growth factor. *J of Neurosci* 2004;12(8):3241–3247. [PubMed: 1494954]
- Hoff SF, Scheff SW, Cotman CW. Lesion-induced synaptogenesis in the dentate gyrus of aged rats: II. Demonstration of an impaired degeneration clearing response. *J of Comp Neurol*. 1993
- Itoh K, Konishi A, Nomura S, Mizuno N, Nakamura Y, Sugimoto T. Application of coupled oxidation reaction to electron microscopic demonstration of horse radish peroxidase: cobalt-glucose oxidase method. *Brain Res* 1979;175(2):341–346. [PubMed: 90544]
- Kamiguchi H, Yoshida K, Sagoh M, Sasaki H, Inaba M, Wakamoto H, Otani M, Toya S. Release of ciliary neurotrophic factor from cultured astrocytes and its modulation by cytokines. *Neurochem Res* 1995;10(10):1187–1193. [PubMed: 8746804]
- Lee MY, Hofmann HD, Kirsch M. Expression of ciliary neurotrophic factor receptor-alpha messenger RNA in neonatal and adult rat brain: an in situ hybridization study. *Neurosci* 1997;77(1):233–246.
- MacLennan JA, Vinson EN, Marks L, McLaurin DL, Pfeifer M, Lee N. Immunohistochemical localization of ciliary neurotrophic factor receptor alpha expression in the rat nervous system. *J Neurosci* 1996;16(2):621–630. [PubMed: 8551346]
- Mallet M, Houlgatte P, Brachet P, Prochiantz A. Lipopolysaccharide-stimulated rat brain macrophages release NGF in vitro. *Dev Biol* 1989;133:309–311. [PubMed: 2707484]
- Moll J. Regeneration of the supraoptico-hypophysial and paraventriculo-hypophysial tracts in the hypophysectomized rat. *Z Zellforschung* 1957;46:686–709.
- Nagata K, Takei N, Nakajima K, Saito H, Kohsaka S. Microglial conditioned medium promotes survival and development of cultured mesencephalic neurons from embryonic rat brain. *J Neurosci Res*. 1993
- Olivieri-Sangiaco C. On the fine structure of perivascular cells in the neural lobe of rats. *Z Zellforschung* 1972;132:25–34.
- Paden CM, Moffet CW, Benowitz LI. Innervation of the rat anterior and neurointermediate pituitary visualized by immunocytochemistry for the growth-associated protein GAP-43. *Endo* 1994;134:503–506.
- Paden CM, Zhou X, Watt JA, Burton R, Pickett J, Oblinger MM. Coordinated upregulation of $\alpha 1$ - and β II-tubulin mRNAs during collateral axonal sprouting of central peptidergic neurons. *J Neurosci Res* 1995;42:402–412. [PubMed: 8583509]
- Perez-Figarez JM, Fernandez-Llebrez P, Marin-Giron F. Presence of microglia-like cells in the neurohypophysis of normal and dehydrated rats. *Acta Anat* 1986;127:195–200. [PubMed: 3788466]
- Pow DV, Perry VH, Morris JF, Gordon S. Microglia in the neurohypophysis associate with and endocytose terminal portions of neurosecretory neurons. *Neurosci* 1989;33(3):567–578.
- Raisman G. Electron microscopic studies of the development of new neurohemal contacts in the median eminence of the rat after hypophysectomy. *Brain Res* 1973;55:245–261. [PubMed: 4714005]
- Reiness CG, Seppa MJ, Dion DM, Sweeney S, Foster DN, Nishi R. Chick ciliary neurotrophic factor is secreted via a nonclassical pathway. *Molecular and Cellular Neuroscience* 2001;17(6):931–944. [PubMed: 11414784]
- Rusnak M, House SB, Gainer H. Long term effects of ciliary neurotrophic factor on the survival of vasopressin magnocellular neurons in the rat supraoptic nucleus in vitro. *J Neuroendoct* 2003;15(10):933–939.
- Rusnak N, House SB, Arima H, Gainer H. Ciliary neurotrophic factor increases the survival of magnocellular vasopressin and oxytocin neurons in rat supraoptic nucleus in organotypic cultures. *Microscopy Research and Technique* 2002;56(2):101–112. [PubMed: 11810713]
- Salm AK, Hatton GI, Nilaver G. Immunoreactive glial fibrillary acidic protein in pituicytes of the rat neurohypophysis. *Brain Res* 1982;236:471–476. [PubMed: 7039766]
- Sendtner M, Kreutzberg GW, Thoenen H. Ciliary neurotrophic factor prevents the degeneration of motoneurons after axotomy. *Nature* 1990;345:440–441. [PubMed: 2342575]
- Siegel SG, Patton B, English AW. Ciliary neurotrophic factor is required for motoneuron sprouting. *Exp Neurol* 2000;166:205–212. [PubMed: 11085886]

- Silverman AJ, Zimmerman EA. Adrenalectomy increases sprouting in a peptidergic neurosecretory system. *Neuroscience* 1982;7(11):2705–2714. [PubMed: 7155347]
- Silverman AJ. Magnocellular neurosecretory system. *Ann Rev Neurosci* 1983;6:357–380. [PubMed: 6301350]
- Steward O. Signals that induce sprouting in the central nervous system: Sprouting is delayed in a strain of mouse exhibiting delayed axonal degeneration. *Exp Neurol* 1992;118:340–351. [PubMed: 1284863]
- Vutskits L, Bartanusz V, Schultz MF, Kiss JZ. Magnocellular vasopressinergic neurons in explant cultures are rescued from cell death by ciliary neurotrophic factor and leukemia inhibitory factor. *Neurosci* 1998;87(3):571–582.
- Watt JA, Bone S, Pressler M, Cranston HJ, Paden CM. Ciliary neurotrophic factor is expressed in the magnocellular neurosecretory system of the rat in vivo: Evidence for injury- and activity-induced upregulation. *Exp Neurol* 2006;197:206–214. [PubMed: 16226750]
- Watt JA, Hobbs NK. Interleukin-1 β immunoreactivity in identified neurons of the rat magnocellular neurosecretory system: Evidence for activity-dependent release. *Exp Neurol*. 1999
- Watt JA, Moffet CW, Zhou X, Short S, Herman JP, Paden CM. Central peptidergic neurons are hyperactive during collateral sprouting and inhibition of activity suppresses sprouting. *J Neurosci* 1999;19(5):1586–1598. [PubMed: 10024346]
- Watt JA, Paden CM. Compensatory sprouting of uninjured magnocellular neurosecretory axons in the rat neural lobe following unilateral hypothalamic lesion. *Exp Neurol* 1991;111:9–24. [PubMed: 1984437]
- Watt JA, Paden CM. Upregulation of the p75 low affinity neurotrophin receptor on active phagocytes during axonal degeneration in the rat neural lobe. *Cell tissue res* 2001;303:81–91. [PubMed: 11236007]
- Widitz D, Philips TM, Bui V, Summy-Long JY. Osmotic stimulation increases IL-1 β release in the supraoptic nucleus of conscious and anesthetized rats. *Society for Neuroscience Abstract* 2003;29:613.9.
- Yan Q, Clark HB, Johnson EM Jr. Nerve growth factor receptor in neural lobe of rat pituitary gland: Immunohistochemical localization, biochemical characterization and regulation. *J of Neurocytol* 1990;19:302–312. [PubMed: 2167948]
- Zambrano D, De Robertis E. The ultrastructural changes in the neurohypophysis after destruction of the paraventricular nuclei in normal and castrated rats. *Z Mikrosk Anat Forsch* 1968;88:496–510.

Figure 1.

Preparations pairing anti-CNTF with antibodies against CR3, p75 low affinity neurotrophin receptor (p75^{LNGFR}), and S100β, demonstrated that CNTF was exclusively localized in a phenotypically-distinct population of perivascular cells. (A) Peroxidase labeling of CNTF in the partially denervated neurohypophysis revealed extensive CNTF-ir in small vellate cells (arrows) closely associated with blood vessels (v). (B) Dual fluorescence labeling showed no colocalization of CNTF-ir (white arrows, Cy-3) with the astrocyte marker S100β (asterisks, FITC). Note the extensive CNTF-ir processes and their close association with blood vessels. (C) Dual peroxidase staining demonstrated no colocalization of OX-42 (arrows, cobalt-DAB) with CNTF-ir (brown, peroxidase). (D–F) Dual fluorescent staining pairing anti-CNTF (D, arrows, FITC) with anti-P75^{LNGFR} (E, arrows, Cy-3), was consistently colocalized with P75^{LNGFR}-ir (F, arrows), although not all P75^{LNGFR}-ir cells were also CNTF-ir (F, asterisk) . (V=blood vessel). Mag bar = 20 μm.

Figure 2.

The association of perivascular cells with phagocytic activity was confirmed by colocalization of CR3-ir and P75-ir in cells expressing the ED-1 antigen. (A) ED-1 profiles (arrows) increased by 3 days post-denervation and were localized within the cytoplasm of OX-42-ir microglial cells. (B) ED-1-ir was also localized within P75LNGFR-ir perivascular cells (arrows); (C) Occasionally, ED-1 was observed in CNTF-ir cells. (D) ED-1 (arrows) was never observed within S100 β -ir pituicytes. (V=blood vessel). Mag bar = 20 μ M.

Figure 3.

Lesion-Induced Increase in CNTF-ir Perivascular Cells. Analysis of group differences (SPSS, V.9.0) confirmed that a significant increase in the number of CNTF-ir cells within the neurohypophysis occurred between 3 and 30 days following partial denervation (1 way ANOVA, $F=2.775$, $df=7, 49$, $P<.018$). The increase in the number of CNTF-ir cells in lesioned animals compared to age-matched sham-operated controls was first significant at 5 days post-denervation and reached a peak at 10 days post-denervation. By 30 days following surgery the mean number of CNTF-ir cells was no longer significantly elevated. Time points and error bars represent the mean and standard error of the mean of 4–10 animals per group. PL indicates number of days post-lesion; Sh indicates age-matched sham control group.