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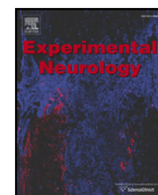

Neuronal activity and axonal sprouting differentially regulate CNTF and CNTF receptor complex in the rat supraoptic nucleus

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ABSTRACT

We demonstrated previously that the hypothalamic supraoptic nucleus (SON) undergoes a robust axonal sprouting response following unilateral transection of the hypothalamo-neurohypophysial tract. Concomitant with this response is an increase in ciliary neurotrophic factor (CNTF) and CNTF receptor alpha (CNTFRα) expression in the contralateral non-uninjured SON from which the axonal outgrowth occurs. While these findings suggest that CNTF may act as a growth factor in support of neuronal plasticity in the SON, it remained to be determined if the observed increase in neurotrophin expression was related to the sprouting response per se or more generally to the increased neurosecretory activity associated with the post-lesion response. Therefore we used immunocytochemistry and Western blot analysis to examine the expression of CNTF and the components of the CNTF receptor complex in sprouting versus osmotically-stimulated SON. Western blot analysis revealed a significant increase in CNTF, CNTFRα, and gp130, but not LIFRß, protein levels in the sprouting SON at 10 days post lesion in the absence of neuronal loss. In contrast, osmotic stimulation of neurosecretory activity in the absence of injury resulted in a significant decrease in CNTF protein levels with no change in CNTFRα, gp130, or LIFRß protein levels. Immunocytochemical analysis further demonstrated gp130 localization on magnocellular neurons and astrocytes while the LIFRß receptor was found only on astrocytes in the SON. These results are consistent with the hypothesis that increased CNTF and CNTFR complex in the sprouting, metabolically active SON are related directly to the sprouting response and not the increase in neurosecretory activity.

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Introduction

Collateral sprouting of axonal processes has been well documented in a variety of neuronal populations within the mature mammalian central nervous system (CNS). However, little is known about the factors that mediate collateral sprouting particularly in regard to the influence of neuronal activity on neurotrophin expression. The magnocellular neurosecretory system (MNS) is comprised of the vasopressinergic (VP) and oxytocinergic (OT) neurons located in the supraoptic (SON), paraventricular (PVN), and accessory hypothalamic nuclei and their projections via the hypothalamo-neurohypophysial tract to the neural lobe (NL) of the pituitary gland. We and others have demonstrated that the MNS provides an excellent model system with which to investigate the cellular mechanisms which underlie activity-dependent axonal reorganization (Morris and Dyball, 1974; Raisman, 1973; Silverman and Zimmerman, 1982; Watt et al., 1999; Watt and Paden, 1991). Toward this end, we utilize a unilateral lesion of the hypothalamo-neurohypophysial tract in which the neurosecretory axons in the animal's right hemisphere PVN and SON are severed while the contralateral nuclei are spared (Watt and Paden, 1991). The lesion results in the loss of 42% of the neurosecretory axons in the NL followed by a return to control levels by four weeks post-lesion (Watt and Paden, 1991). The axonal recovery results from a collateral sprouting response arising from the non-injured, contralateral magnocellular neurons with a concomitant increase in; the magnocellular neuron somatic and nuclear area, oxytocin and vasopressin mRNA expression (Watt and Paden, 1991), and alpha-I and beta-II tubulin mRNA expression (Paden et al., 1995). Daily measures of urine osmolality reveal a chronic hyperosmolality with a concomitant decrease in daily water intake and urine excretion volume, which persists throughout the post-surgical period. Together these results indicate that the sprouting event is not a compensatory response as it occurs in the absence of a functional deficit (Watt and Paden, 1991). Hence, the mechanism underlying the axonal sprouting remains undetermined.

Abbreviations: CNTF, ciliary neurotrophic factor; CNTFRα, ciliary neurotrophic factor receptor alpha; LIFRß, leukocyte inhibitory factor receptor beta; SON, supraoptic nucleus; PVN, paraventricular nucleus; VP, vasopressinergic; OT, oxytocinergic; NL, neural lobe; ROD, relative optical density; GFAP, glial fibrillary acidic protein; VGL, ventral glial limitans; BDNF, brain derived neurotrophic factor; FGF, fibroblast growth factor; NGF, nerve growth factor.

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Collateral sprouting has been shown to occur in a variety of neuronal populations; however, the factor or factors responsible for mediating the sprouting response are still largely ill defined. Ciliary neurotrophic factor (CNTF) has been implicated in hypothalamic magnocellular neuron sprouting in vitro (Vutskits et al., 1998) and has been demonstrated to promote motor neuron sprouting (Gurney et al., 1992; Guthrie et al., 1997; Kwon and Gurney, 1994; Oyesiku and Wigston, 1996; Siegel et al., 2000; Simon et al., 2010; Ulenkate et al., 1994; Wright et al., 2007; Xu et al., 2009). CNTF signals through the tripartite receptor complex consisting of the specific receptor for CNTF, CNTF receptor alpha (CNTFRα), and the gp130 and LIFRß receptor subunits, to promote the survival of multiple neuronal phenotypes affected by injury across numerous species (Albrecht et al., 2002; Arakawa et al., 1990; Ip et al., 1991; Larkfors et al., 1994; Lehwalder et al., 1989; Magal et al., 1991; Sendtner et al., 1990). Moreover, CNTF is a potent promoter of hypothalamic magnocellular neuron survival in vitro (House et al., 2009; Rusnak et al., 2002, 2003; Vutskits et al., 1998, 2003). In our studies we have demonstrated an increase in CNTF-immunoreactivity (Watt et al., 2006) and CNTFRα mRNA expression (Watt et al., 2009) within the non-injured contralateral SON, which contains the sprouting and metabolically active magnocellular neurons. Together, these observations suggest that CNTF contributes to the axonal sprouting response. However, it remained to be determined if the observed increases CNTF and CNTFRα in the contralateral SON were related to the sprouting response per se or more generally to the increased neurosecretory activity associated with the post-lesion response. Therefore, the aim of the present study was to test the hypothesis that the observed increase in CNTF and CNTFRα in the SON contralateral to the unilateral lesion is related specifically to the onset of collateral axonal sprouting and not to the increase in neurosecretory activity which occurs concurrently with the sprouting event.

Experimental procedures

Animals

Male Sprague–Dawley rats were purchased from Charles River Laboratories (Wilmington, MA) and housed in the University of North Dakota Center for Biomedical Research Facility, an AAALAC accredited facility, under a 12L:12D light cycle with ad lib access to lab chow and tap water throughout the investigations unless otherwise noted. Experimental protocols utilized in these studies followed the guidelines in the NIH guide for the care and use of laboratory animals and were approved by the UND Institutional Animal Care and Use Committee. Animals were 35–40 days of age (75–100 g) at the time a unilateral hypothalamic knife cut of the hypothalamo-neurohypophysial tract was performed. The animals were secured in a stereotaxic apparatus (Stoelting, Wood Dale, IL) and kept under constant isoflurane anesthesia (2.5%; Abbot Laboratories; Abbott Park, IL) using a tabletop anesthesia apparatus (Matrx Quantiflex Low Flow V.M.C.; Matrx, Orchard Park, NY) equipped with an isoflurane Vaporizer (Matrx VIP 3000; Matrx). A wire knife constructed of HTX-33-gauge tubing was used to unilaterally transect the entire length of the hypothalamus through which the hypothalamo-neurohypophysial tract passes as previously described (Watt and Paden, 1991). The knife tract extended from the dorsal to the ventral surface of the brain, medial to the ipsilateral SON, but passing through the lateral aspect of the ipsilateral PVN. Stereotaxic lesion coordinates were 0.6 mm lateral to the midsagittal suture, and the lesion extended from −4.0 mm to +4.0 mm anterior–posterior from bregma. This results in complete transection of the ipsilateral hypothalamo-neurohypophysial tract. The animals were sacrificed 10 days post surgery along with age-matched non-injured control animals. All lesion tacks were confirmed histologically using cresyl violet. Only animals with a complete transection of the hypothalamo-neurohypophysial tract were included in these studies.

Male Sprague–Dawley rats (200–250 g) in the chronic salt-loaded groups were given 2% salt water substituted for tap water for 10 days prior to sacrifice. The animals were sacrificed with age-matched control animals. All efforts were made to minimize the numbers of animals used in this study and their suffering.

Gel electrophoresis and Western blot analysis

Following experimental periods, the animals were anesthetized with isoflurane, decapitated, and their brains were removed intact. SON samples were carefully collected under a dissecting microscope and pooled from 6 rats (30 total rats, n = 5 groups of 6 pooled rat SON) in a solution of radioimmuno-precipitation assay (RIPA) buffer containing 20 mM Tris (pH 7.5 Sigma; St. Louis, MO), 150 mM NaCl (Sigma), 1% nonidet P-40 (Roche Diagnostics; Indianapolis, IN), 0.5% sodium deoxycholate (Sigma), 1 mM EDTA (Sigma), 0.1% SDS (Pierce; Rockford, IL), 1% protease inhibitor (Protease Inhibitor Cocktail; Sigma) and 1% phosphatase inhibitor (Phosphatase Inhibitor Cocktail 2; Sigma). The SON samples were then homogenized in RIPA buffer and centrifuged at 10,000 rpm for 20 min at 4 °C. Supernatant from each sample was stored at −80 °C until needed. SON protein content was determined using the bicinchoninic acid (BCA) colorimetric detection assay (Pierce BCA Protein Assay; Pierce). Each lane was loaded with 50 μg of protein and separated by a 12% SDS-PAGE gel (Precise Protein Gels; Pierce) at 90 V for approximately 1.5 h and then electrophoretically transferred to a PVDF membrane (0.2 μm; Bio-Rad, Hercules, CA) at 70 V for 2 h. After blocking non-specific binding sites (5% nonfat milk in phosphate-buffered saline (PBS) plus 0.1% Tween-20; blocking buffer; Bio-Rad), the membranes were incubated overnight at 4 °C in rabbit anti-CNTF (1:5000; #AAR21, Serotec, Raleigh, NC). The membranes were then washed repeatedly for 1 h in PBS-Tween and incubated for 2 h in the appropriate HRP-conjugated secondary antibody (1:100,000; Santa Cruz Biotechnology, Santa Cruz, CA). Following PBS washes for 2 h, the bands were subsequently visualized using the West Femto chemiluminescent detection kit (Pierce) with high performance chemiluminescence film (Amersham Hyperfilm ECL; GE Healthcare; VWR; West Grove, PA) on an AGFA CP1000 film processor (DMS Health Group; Fargo, ND). Subsequently, bound antibodies were removed with stripping buffer (pH 2.2; 15 g glycine; Sigma, 1 g SDS; Bio-Rad, 10 ml Tween-20; Bio-Rad in 1 l ultrapure water) for 10 min and the steps were repeated to sequentially reprobe the membrane for the following antibodies; mouse anti-CNTFRα (1:20,000; #558783, BD Biosciences, Franklin Lakes, NJ), rabbit anti-gp130 (1:5000; #sc-655, Santa Cruz Biotechnology), rabbit anti-LIFRß (1:5000; #sc-659, Santa Cruz Biotechnology), and mouse anti-ß-actin (1:50,000; #A2228, Sigma).

Densitometric analysis of immunoblot signals was performed using MCID image analysis software (Version 7.0, Imaging Research Inc.). Briefly, digitized Western blot films were opened in MCID, bands of interest were outlined, with band area and relative optical densities (ROD) then determined. The area of the band was then multiplied by the density value. The ROD of all bands was normalized to the respective ROD of ß-actin bands to obtain ratios. Analysis was repeated on 3 separate samples per group resulting in mean ratio values for each group that were used for statistical analysis as described below.

Immunocytochemistry

Animals were deeply anesthetized with isoflurane and perfused transcardially with 0.9% saline followed by periodate-lysine-paraformaldehyde fixative (PLP; 3.2% paraformaldehyde, 2.2% lysine, 0.33% sodium-(meta) periodate; Sigma) prepared immediately before use (McLean and Nakane, 1974). Brains were then removed and post-

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