Journal of Neuroscience Research 37:278–286 (1994)

Rapid Communication

Increased CNTF Gene Expression in Process-Bearing Astrocytes Following Injury Is Augmented by R(−)-Deprenyl

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R(−)-deprenyl has been shown to rescue axotomized immature facial motoneurons with an efficacy comparable to that of the neurotrophic factors CNTF and BDNF (Salo and Tatton, J Neurosci Res 31:394–400, 1992; Ansari et al., J Neurosci 13:4042–4053, 1993). Recent work has suggested that some of the actions of (−)-deprenyl may be mediated through reactive astrocytes (Biagini et al., NeuroReport 4:955–958, 1993). To test this proposal we have developed an in vitro model of reactive gliosis consisting of a mixed astrocyte population of flat and process-bearing (PB) astroglia taken from postnatal day (PD) 2 or PD5 rat cerebral cortex. After mechanical wounding, PB astrocytes preferentially migrate into the wound zone while flat astrocytes maintain their position at the wound edge. CNTF mRNA was localized to PB astrocytes, but not flat astrocytes, as determined by in situ hybridization using biotin-labelled riboprobes. Following "wounding," there was an increase in CNTF mRNA in PB astrocytes only, which could be further enhanced by a single pulse of (−)-deprenyl (10^{-8}–10^{-11} M) 48 hr after injury. (−)-Deprenyl also increased the total process length of PB astrocytes after wounding by an average of 50%. The stereoisomer (+)-deprenyl (10^{-9} M) had no effect on either astrocyte process length or CNTF mRNA content. This is the first report to our knowledge of an agent which can upregulate CNTF gene expression in astroglial cell culture as well as influence glial cell process length. We propose that some of the trophic-like actions of (−)-deprenyl may be mediated through a specific subpopulation of astroglia.

Key words: CNTF mRNA, deprenyl, in situ hybridization, astrocytes, injury

INTRODUCTION

Clinical trials have demonstrated that R(−)-deprenyl delays the need for levodopa treatment in Parkinson's disease (Parkinson Study Group, 1993) and slows the progression of some neuropsychological deficits in Alzheimer's disease (Mangoni et al., 1991). The basis for these actions in Parkinson's disease and Alzheimer's disease is unknown and controversial (Schulzer et al., 1992). (−)-Deprenyl has also been shown to significantly reduce the death of motoneurons following axotomy of the facial nerve in postnatal day (PD) 14 rats (Salo and Tatton, 1992; Tatton, 1993). The ability of parenterally administered (−)-deprenyl to rescue motoneurons compares favourably to the rescue achieved by the neurotrophic factors CNTF and BNDF (Sendtner et al., 1990, 1992; Yan et al., 1992), which were applied directly to the severed end of the facial nerve. (−)-Deprenyl also markedly increased dopaminergic neuron survival after MPP+ damage (Tatton and Greenwood, 1991). At present the mechanism by which deprenyl can rescue neurons after axotomy or neurotoxin-induced injury is unknown. Importantly, increased neuronal survival was achieved at doses too small to inhibit the enzyme monoamine oxidase B (Ansari et al., 1993; Tatton et al., 1993). The rescue capacity of (−)-deprenyl was stereospecific, since the stereoisomer (+)-deprenyl did not rescue even at high doses.

Astroglial hypertrophy in response to axotomy is well documented, especially the capacity of astrocytes to elaborate processes which surround the axotomized neurons in a lamellar-like fashion (Graeber and Kreutzberg,

Received September 27, 1993; revised November 2, 1993; accepted November 4, 1993.

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1986, 1988). Although this intimate contact can exist for months after the lesion, its immediate function is unclear. Systemic administration of (−)-deprenyl has been shown to enhance glial hypertrophy, as measured by increased GFAP immunoreactivity, in response to traumatic injury in vivo (Biagini et al., 1993). Astroglial hypertrophy may also involve increased CNTF gene expression since in vivo mechanical wounding demonstrated increased CNTF mRNA by in situ hybridization in astroglia in the immediate wound zone (Ip et al., 1993).

We have created an in vitro model of reactive gliosis with a heterogeneous culture of flat (also known as type 1) and process-bearing (also known as type 2), O2A-lineage astrocytes in order to examine the actions of (−)- and (+)-deprenyl on astrocytes, independent of any neuronal interaction. Astrocyte process length and CNTF mRNA expression following wounding and/or (−)-deprenyl treatment of these astrocyte cultures were quantitated using computer-assisted methods. We found that 1) PB astrocytes migrate toward the wound zone; 2) only PB astrocytes contain detectable CNTF mRNA in this mixed culture model; 3) wounding alone stimulates detectable increases in the expression of CNTF mRNA in PB astrocytes only; 4) (−)-deprenyl is able to upregulate CNTF mRNA in PB astrocytes when given 48 hr after the injury; and 5) (−)-deprenyl can increase the process length of those PB astrocytes in the wound zone.

MATERIALS AND METHODS

Astrocyte Cultures

Primary astrocytes were obtained from Sprague-Dawley rat pups, PD2 or PD5, following a modification of the selective adhesion protocol of McCarthy and de Vellis (1980) and maintained in DME/F12 media containing 15 mM HEPES (containing 10% fetal bovine serum (FBS), 2% horse serum, 50 units/ml penicillin, 50 μg/ml streptomycin, and 0.1 mg/ml fungizone). Flasks were placed on an orbital shaker at 37°C after cells reached confluency and were agitated until the density of the phase dark layer of cells was reduced, usually 5–7 days. A mixed population of flat and PB astrocytes was plated at high density on poly-lysine coated glass coverslips (Assistent Glass, Carolina Biological Supply Co.) and allowed to stabilize for 3–5 days in media containing 5% FBS. Astrocytes had been in vitro approximately 21 days by the start of the wounding experiment. Using a template guide, a reproducible small wound, approximately 20% of the total area, was created by scraping a central square of cells from the coverslip at t = 0 hr. The media was replaced at t = 48 hr with fresh media alone or media supplemented with (−)-deprenyl (10^{-8}–10^{-11} M) or (+)-deprenyl (10^{-9}, 10^{-11} M). Cells were fixed at t = 72 hr.

Immunocytochemistry

Cells on the coverslips were fixed for 10 min at room temperature with 4% paraformaldehyde and pretreated with methanol at −20°C for 10 min before blocking with 10% normal horse serum. Primary monoclonal antibody incubations with vimentin (Boehringer Mannheim, 814 318), GFAP (Sigma, G 3893), or proliferating nuclear cell antigen (PCNA, Boehringer Mannheim, 1486 772) were for 1 hr at 37°C. Coverslips were incubated with biotinylated horse anti-mouse IgG (Vector Labs) for 40 min, followed by a 30 min incubation with avidin-HRP (Elite kit, Vector Labs). Diaminobenzidene (1.2 mg/ml) mixed v:v 0.02% hydrogen peroxide was used for chromogenic detection. A few coverslips were incubated with an affinity purified polyclonal antibody to CNTF peptide (see Henderson et al., 1994 for details) to confirm the presence of CNTF in the astrocytes.

In Situ Hybridization

In situ hybridizations were performed on coverslips using antisense and sense riboprobes of full length rat CNTF cDNA transcribed and biotin-labelled using a non-isotopic labelling kit (Gibco, 8093SA). G50 column-purified probes were run on a 1% agarose gel against an RNA ladder and a biotin-labelled standard. Probes were used at an approximate concentration of 400 ng/ml. Hybridization buffer included probe plus 50% formamide, 4 × SSC (20 × SSC = 3 M NaCl, 0.3 M NaCitrate), 10 mg/ml dextran sulfate, 1 mg/ml yeast tRNA, and 1 × Denhardt's. Hybridization was at 42°C for 3 hr, followed by several high stringency washes. Following avidin-HRP incubation, control and experimental series were run simultaneously and carefully timed for development of the colour reaction (see Seniuk et al., 1990 for details) to allow for optical density (OD) measurements (Tatton et al., 1990).

Image Analysis

We have previously described a method for measuring the OD of the DAB-peroxidase reaction product as a relative measure of mRNA:antisense probe hybridization complex (Seniuk et al., 1991). Images taken with a Javelin MOS camera on a Polyvar 2 microscope (Leitz) were digitized and analyzed using a Matrox frame grabber and Metamorph software (Universal Imaging, W. Chester, PA). A calibrated box was placed over each randomly selected PB astrocyte soma and the OD measured within the box (n = 50 cells). Each somal OD was background corrected using the average value of two ODs from boxes placed immediately outside each PB astrocyte soma. The corrected values are referred to as the 'cytoplasmic' OD. Primary (those processes originating from the cell soma) process length (n = 50) was

measured using a predetermined calibration square to convert pixel length to microns. Measurements of secondary processes (those originating from a primary process and not the soma) were taken only for astrocytes from PD5 cultures. Non-parametric statistical analysis (Siegel, 1956) was performed using Statistica software (StatSoft, Tulsa, OK).

RESULTS

Both flat and PB astrocytes were immunoreactive for GFAP and vimentin. PB astrocytes from PD5 cortex are shown growing on a bed layer of flat astrocytes prior to wounding. The PB astrocytes characteristically demonstrated more intense immunoreactivity for both intermediate filament proteins. Note the extensive processes of the PB astroglia and the large, round eccentric nucleus (Fig. 1A). Most PB astrocytes in the wound zone were positive for CNTF (Fig. 1B) with strong immunoreaction in the nucleus and less dense immunoreactivity in the cytoplasm, while those PB astrocytes distant from the wound zone displayed less immunoreactivity. PB astrocytes were only lightly CNTF immunopositive in unwounded cultures (data not shown). This corresponded with our observations on the hybridization density of CNTF mRNA (Fig. 2). There was no detectable CNTF immunoreactivity in the flat astrocyte bed layer.

Following wounding, PB astrocytes migrated into the cleared central wound zone, while the flat astrocytes tended to maintain their relative position at the wound edge and extended short, pseudopod-like processes into the cleared area. This spreading appeared to be directed toward reattachment rather than migration into the wound zone, particularly when compared with the massive movement of PB astrocytes. Figure 1C shows a wounded culture from PD5 cortex at t = 72 hr clearly showing vimentin immunopositive PB astrocyte migration into the cleared wound area and flat astrocytes forming the wound 'edge' at the top of the photomicrograph. An identical wounded culture treated with 10^{-8} M (−)-deprenyl at t = 48 hr (Fig. 1D) illustrates the increase in processes of PB astrocytes compared to the media-fed wounded cultures. PB astrocytes from PD2 cultures were also immunoreactive for CNTF and were able to migrate into the cleared wound zone. However, these astrocytes did not appear as completely differentiated as the PB astrocytes from PD5 cortex, having fewer processes per cell and remaining at a higher density (data not shown).

In situ hybridizations with biotinylated riboprobes for CNTF mRNA are pictured in Figure 2. Interference contrast microscopy was used to allow visualization of PB astrocytes and flat astrocytes particularly for the sense probe hybridizations which were otherwise invisible to the camera (Fig. 2A). CNTF antisense hybridizations of unwounded cultures (Fig. 2B) demonstrated CNTF mRNA exclusively in the PB astrocytes. There was no detectable hybridization signal in the flat astrocytes. Following wounding, CNTF mRNA appeared to have increased in the PB astrocytes as gauged by the increased density of chromogenic reaction product (Fig. 2C). No CNTF hybridization signal was detectable in the flat astrocytes after wounding. In Figure 2D, a wounded culture treated with 10^{-8} M (−)-deprenyl at t = 48 hr shows increased CNTF mRNA hybridization signal as compared to the media-only wounded culture (Fig. 2C).

OD measurements for CNTF mRNA hybridization signal in PB astrocytes from PD5 cortex are presented in Figure 3A. All background and cytoplasmic ODs were normalized to the mean sense background OD. The background OD values from each group, presented as black histogram bars, demonstrate that the backgrounds were essentially identical for all treatment groups, allowing comparison of the PB cytoplasmic ODs. The sense probe ODs (Fig. 3A1) are slightly more dense than the background since they are taken from PB cells sitting on a bed layer of astrocytes. Wounded cultures (Fig. 3A2) had significantly greater hybridization signal ODs at t = 72 hr as compared to the sense probe ODs ($P < .007$). There was no significant difference in cytoplasmic ODs between the 10^{-11} M (+)-deprenyl wounded cultures (Fig. 3A3) at t = 72 hr as compared to the wound only cultures ($P < .341$). However, the 10^{-11} M (−)-deprenyl wounded cultures at t = 72 hr had significantly increased OD values for the antisense CNTF probes as compared to the wound only cultures ($P < .012$). In order to confirm that the increased OD values were not merely the result of a decrease in cell soma size, the cross-sectional somal areas of PB astrocytes in 10^{-8} M (−)-deprenyl (Fig. 3B1) and media-only (Fig. 3B2) cultures were compared. There was no significant difference ($P < .321$) in the cross-sectional somal areas between the two treatment groups, indicating that increases in hybridization signal density were unlikely to be due to a reduction in cell size.

Figure 4 illustrates cytoplasmic OD measurements of CNTF mRNA hybridization signal for astrocytes from PD2 cortex treated over a dosage range of 10^{-8}–10^{-11} M (−)-deprenyl. OD values were normalized to the mean sense background OD value. The data is presented as box plots so that the distributions across cell populations are readily observed. Note that the relative background ODs of the individual treatments are essentially identical to the sense background. There was a significant increase in the OD values of wounded cultures at t = 72h with increasing (−)-deprenyl dosage as compared to the media-fed wound only culture ($P < .001$, 10^{-11}–10^{-8} M). Treatment with 10^{-9} M (+)-deprenyl after wounding did not result in any significant difference in hybridiza-

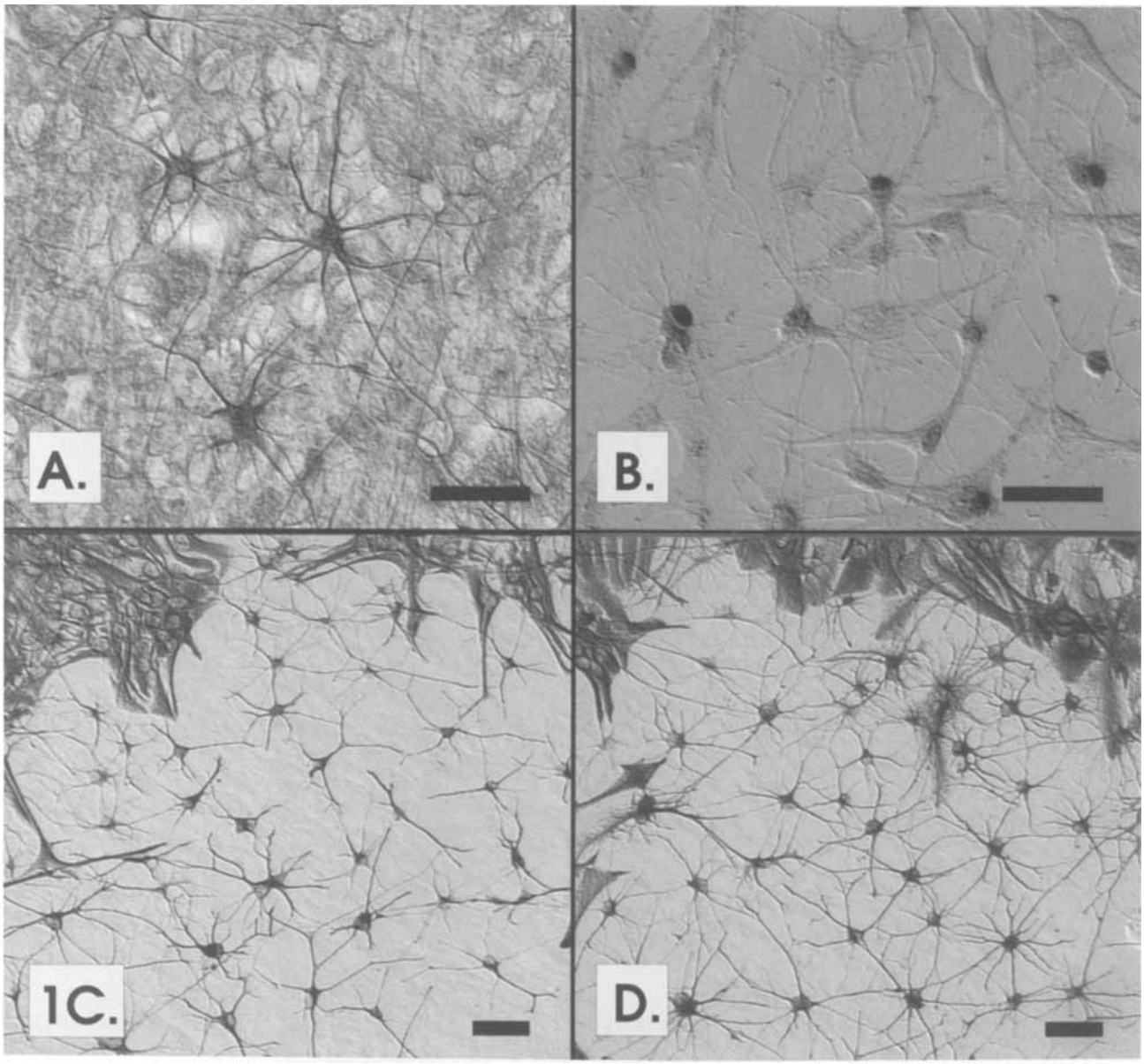

Fig. 1. PB astrocytes and flat astrocytes in the wound model. **A:** Unwounded PD5 culture showing GFAP immunopositive PB astrocytes on a bed layer of GFAP immunopositive flat astrocytes. **B:** CNTF immunopositive PB astrocytes from PD2 cultures in the wound zone. Note the dense CNTF immunoreactivity in the nucleus of the PB astroglia with only light immunoreactivity in the PB astrocyte cytoplasm. Most PB glia in the wound zone are CNTF immunopositive. Bar = 20 μm for A,B. **C:** Wounded PD5 culture with vimentin immunopositive PB astrocytes in the cleared wound zone. Flat astrocytes at the top of the micrograph forming the wound edge show evidence of spreading but not migration. Both flat and PB astrocytes are vimentin immunopositive. **D:** An identical culture treated with 10^{-8} M (−)-deprenyl at t = 48 hr. Note the elaborate process development of the PB glia, and that flat astrocytes largely maintain their position and extend only short, broad processes into the cleared zone. Bar = 50 μm for C,D.

tion signal OD when compared to the media-fed wound-only cultures. Cytoplasmic OD values for CNTF hybridization signal for PB astrocytes from unwounded cultures treated with (−)-deprenyl were not significantly increased as compared to the media-fed unwounded culture, indicating that (−)-deprenyl alone did not upregulate CNTF gene expression. CNTF immunoreactivity was not quantitated for unwounded, (−)-deprenyl treated cultures which did not appear to show an increase in CNTF hybridization signal. Therefore, wounding alone

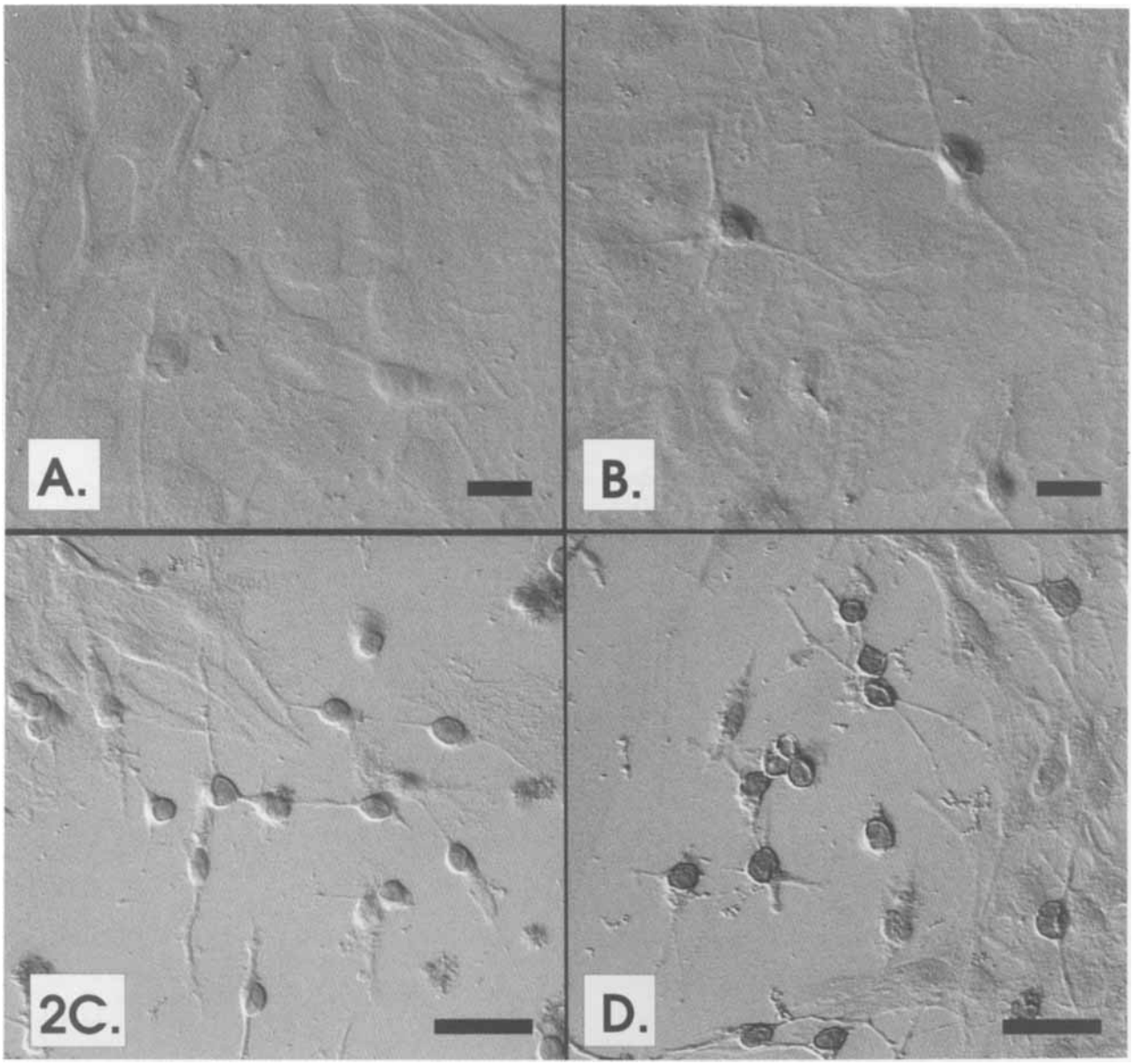

Fig. 2. CNTF mRNA localized to PB astrocytes by in situ hybridization. **A:** Sense probe control hybridization of unwounded culture at t = 72 hr. Note the lack of detectable signal in PB or flat astrocytes. Interference contrast was used to enhance the edges of cells to visualize their processes for identification. **B:** CNTF antisense probe hybridization at t = 72 hr on an unwounded culture. Note that the hybridization signal is detectable only in PB astrocytes and not flat astrocytes. **C:** CNTF antisense probe hybridization at t = 72 hr on a wounded culture. Note the increased hybridization signal in the PB astrocytes as compared to B, while there is still no detectable signal in the flat astrocyte bed layer. **D:** CNTF antisense probe hybridization at t = 72 hr on a wounded culture, exposed to 10^{-8} M (−)-deprenyl at t = 48 hr. Note the increased hybridization signal when compared with C, and that there is no detectable signal in the flat astrocytes. Bar = 20 μm.

could stimulate CNTF gene expression, but wounding followed by (−)-deprenyl provided an even greater stimulus to increase CNTF gene expression. Therefore, PB astrocytes must be in an 'activated' state to permit induction of CNTF gene expression by (−)-deprenyl.

Measurements of primary, secondary, and total process lengths for 10^{-8} M (−)-deprenyl treated wounded cultures and wound only cultures from PD5 cortex are plotted as histograms in Figure 5. (−)-Deprenyl treatment significantly increased the total process length (primary plus secondary processes) by approximately 50% ($P < .009$). Total primary process length for

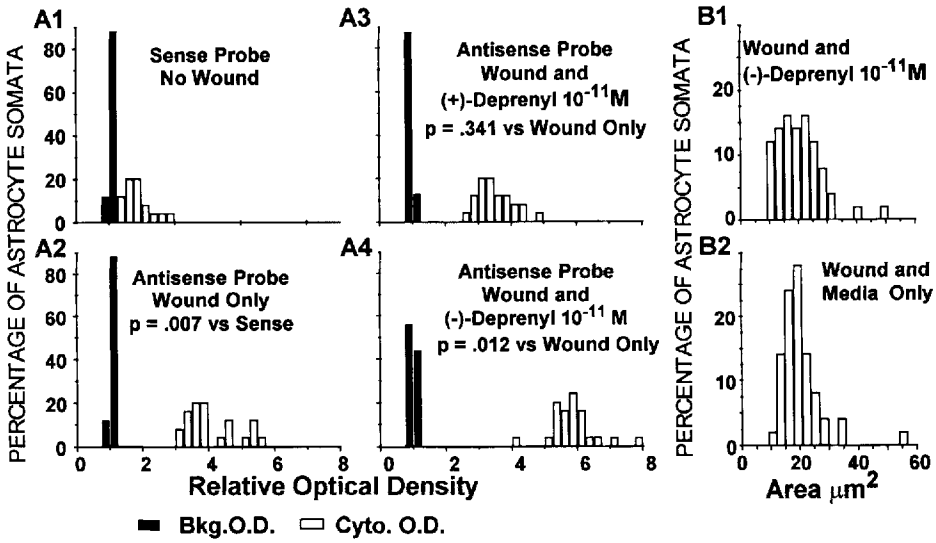

Fig. 3. Frequency distribution histograms of CNTF relative OD measurements in PB astrocytes from PD5 cortex. **A1–A4:** All OD values are normalized to the mean background OD of the sense hybridization. Black bars represent relative background for each treatment group and are identical to the sense background (A1). White bars are 'cytoplasmic' ODs which have been corrected for background (see Materials and Methods). **A1:** The sense control probe hybridizations appear slightly higher than background due to the PB astrocyte ODs being measured through a bed layer of astrocytes. **A2:** Antisense CNTF hybridization at t = 72 hr after wounding. Note that the hybridization signal is significantly greater than the sense hybridization. **A3:** Antisense hybridization at t = 72 hr after wounding and (+)-deprenyl at t = 48 hr. There was no significant change in OD as compared to the wound-only astrocytes in A2. **A4:** Antisense hybridization at t = 72 hr and (−)-deprenyl at t = 48 hr. OD values were significantly increased as compared to wound only OD values in A2. **B1–B2:** Histograms of the somal area of PB astrocytes from (−)-deprenyl and media-fed wounded cultures indicate no change in the somal area of PB astrocytes.

PB astrocytes from PD2 cortex did not demonstrate any significant increase at t = 72 hr with (−)-deprenyl treatment following wounding but at t = 120 hr process length had increased by approximately 45% ($P < .006$). The average number of processes per PB astrocyte did not change with 10^{-8} M (−)-deprenyl for either PD2 (5.94 ± 1.45, media; 6.08 ± 1.94, deprenyl) or PD5 ($P = .06$) astrocytes (6.9 ± 2.2, media; 8.2 ± 2.4, deprenyl) at t = 72 hr. Accordingly, (−)-deprenyl appeared to influence process length but not the branching of the processes or the number of processes per astrocyte.

DISCUSSION

We have shown that CNTF mRNA is localized to PB astrocytes and that flat astrocytes do not have detectable (by in situ hybridization) levels of CNTF mRNA in a mixed culture from either PD2 or PD5 rat cortex. Within 72 hr after the initial injury, CNTF mRNA was significantly elevated in PB astrocytes but was still not detectable in flat astrocytes. (−)-Deprenyl given 48 hr after wounding enhanced CNTF gene expression already increased by wounding. Further, we found that (−)-deprenyl increased the total process length of PB astrocytes from PD5 cortex at t = 72 hr of wounding, but did not significantly alter the process length of PD2 cortex astrocytes until t = 120 hr. Both actions of (−)-deprenyl were stereospecific in that (+)-deprenyl had no effect on PB astrocyte process length or CNTF gene expression.

We cannot determine from these experiments whether increased astrocyte process length is a direct action of (−)-deprenyl or whether the changes in cytoskeletal protein polymerization and/or synthesis may be due to factors synthesized by the deprenyl-stimulated astrocytes such as basic fibroblast growth factor (bFGF) or CNTF. It has been demonstrated in vivo in the rodent CNS that injection of basic FGF can enhance astroglial scar formation after an electrolytic lesion (Eclancher et al., 1990).

Astrocytes from wounded PD2 cortex cultures did not show an increase in process length after deprenyl until t = 120 hr. These PB astrocytes did not appear as well differentiated upon initial plating when compared to their counterparts in the PD5 cultures. Specifically, there were fewer processes per cell, so that these astrocytes were more reminiscent of O2A progenitors than of fully differentiated PB astrocytes. However, by t = 120 hr,

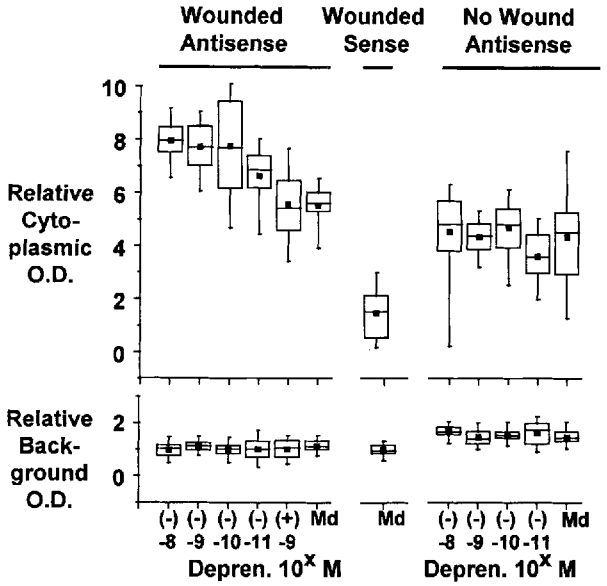

Fig. 4. Box plots of CNTF relative OD measurements in PB astrocytes from PD2 cortex. Box plots were used to show distribution of cytoplasmic CNTF OD for the dosage range of (−)-deprenyl stereoisomer indicated by (−) above the molarity (−8 to −11) for wounded and unwounded cultures. The (+)-deprenyl stereoisomer is indicated by (+) above the molarity (−9), while media-only treatment is indicated by Md. The box alone comprises 50% of the distribution of OD values, while the median is indicated by the bar and the mean by the black square within the box. The lower and upper error bars include the range of OD values from 10% to 90%. OD values were normalized to mean sense background OD as in Figure 3. Note that (−)-deprenyl alone did not upregulate CNTF expression of PB astrocytes in the unwounded cultures. Wounding alone did increase CNTF gene expression (media only), and wounding followed by (−)-deprenyl further augmented CNTF mRNA over a dosage range of 10^{-11}–10^{-8} M.

these astrocytes more closely resembled the activated PD5 glia at t = 72 hr. This may be attributable to their earlier harvesting from the primary tissue and thereby being less differentiated, since all other procedures were identical.

By using this mixed astrocyte culture model, we may have created a different cell-cell signalling environment from that found in primary cultures of either flat or PB astrocytes alone. It has previously been reported that type 2/PB astrocytes contain about one-half the GFAP content of type 1/flat astrocytes under "normal" growth conditions (Levison and McCarthy, 1991), yet we consistently observed greater GFAP immunoreactivity in the PB astrocytes in our unwounded cultures (see Fig. 1A). This may be the result of an apparently higher concentration of GFAP per unit volume due to the compact processes of the PB astrocytes as compared with the broader flat astrocytes.

Others have reported that a subpopulation of type 1 astrocytes synthesizes CNTF (Stockli et al., 1989, 1991; Lillien and Raff, 1990), which is apparently in conflict with our data. These studies relied upon Northern blot analysis to detect CNTF mRNA rather than in situ hybridization. The discrepancy may be due to different environmental signals present in our mixed culture model when compared with the relatively homogeneous cultures. We are currently examining both heterogeneous and homogeneous cultures of flat and process-bearing astrocytes by in situ hybridization and RNAse protection analysis to determine whether in our wound model, flat astrocytes down-regulate CNTF gene expression. Although our process-bearing astrocytes morphologically resemble type 2 astrocytes described by others (Ingraham and McCarthy, 1989; Levison and McCarthy, 1991) using homogeneous culture techniques we are in the process of establishing their lineage using antigenic markers.

At present the identity of the astrocyte subpopulation(s) which can participate in the reactive gliotic response in vivo is unknown. Our initial attempts at creating an in vitro wound model resembled those of Yu and his colleagues (Yu et al., 1993) using a relatively pure culture of flat astrocytes. Although we also noted increased GFAP immunoreactivity in those flat astrocytes at the wound edge, in our hands the flat astrocyte culture did not yield significant migration to the wound site or changes in CNTF expression, which appeared to be important features of in vivo wounding (Ip et al., 1993; Janeczko, 1993). Using a heterogeneous astrocyte population we observed a response which appeared to more closely resemble the in vivo response to mechanical wounding with respect to CNTF expression and migration of vimentin positive glia to the wound site. Recent efforts examining reactive gliosis in vivo (Trimmer, 1993; Schiffer et al., 1993; Schmidt-Kastner et al., 1993; Petito and Halaby, 1993) have demonstrated that astrocytes are immunoreactive for both vimentin and GFAP but have not yet established the identity of the responsive astroglia. Although it has been reported that native astrocytes may not migrate following local trauma (Hatton et al., 1993) this issue is controversial.

It has been reported that CNTF produced by type 1 astrocytes is required for differentiation of type 2 astrocytes using rat optic nerve cultures (Lillien et al., 1988; Lillien and Raff, 1990). A recent report on the CNTF knockout transgenic mouse indicates that this is not a fatal knockout and that the animals appeared normal at 4 weeks of age (Masu et al., 1993), suggesting that CNTF is not an absolute requirement in the development of O2A lineage cells and that other factors may function interchangeably with it. Interestingly, the glial response to injury in these mice appeared to be dominated by microglia. Given our findings this suggests that either

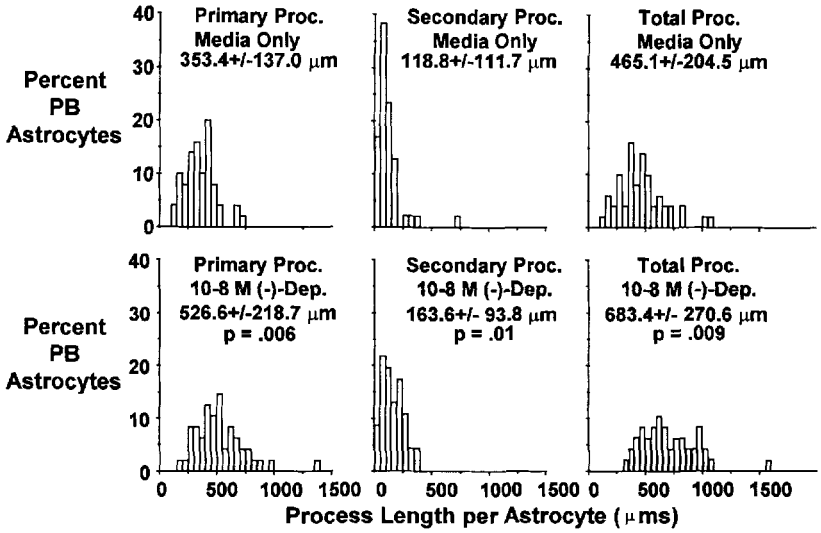

Fig. 5. Distribution histograms of PB astrocyte process lengths from PD5 cortex. Mean and standard deviation are given for each treatment group. (−)-Deprenyl treatment significantly increased total process length by approximately 50% as compared to media only cultures at t = 72 hr after wounding.

complete differentiation of a "type 2" astrocyte did not occur and that these cells are an integral part of the gliotic response in vivo or that CNTF may be required for reactive astrocyte gliosis (but not microgliosis).

We have shown that (−)-deprenyl is able to upregulate CNTF mRNA in activated PB astrocytes, but not quiescent PB astrocytes. To the best of our knowledge (−)-deprenyl is the first agent which has demonstrated an ability to unpregulate CNTF gene expression. Clearly, cellular events related to defining a reactive astrocyte also enable the astrocyte to respond to deprenyl. Although we have quantitated changes in CNTF expression and astrocyte process length, deprenyl may also elicit other changes in reactive astroglia. The ability of (−)-deprenyl to stimulate gene expression is not restricted to astrocytes in culture or to CNTF. Others have shown that (−)-deprenyl stimulates the expression of aromatic amino acid decarboxylase (EC 4.1.1.28) in PC12 cells in vitro (Li et al., 1992), while it can increase glial cell hypertrophy after injury in vivo (Biagini et al., 1993). Rats treated with (−)-deprenyl have recently been shown to significantly increase trkC mRNA levels in the neocortex (Ekblom et al., 1993). We suggest that (−)-deprenyl may enhance the reactive gliosis response following axotomy by selective upregulation of at least one neurotrophic factor, CNTF, and by facilitating process formation in at least one astroglial subpopulation. We believe that these actions of (−)-deprenyl on astroglia can occur in parallel with direct actions of (−)-deprenyl on neurons. Finally, it has been observed that (−)-deprenyl can delay apoptosis in serum-deprived PC12 cells in vitro (K. Ansari and W. Tatton, personal communication). This would suggest that the role of astrocytes in supporting the recovering neuron may reside in providing an appropriate milieu by virtue of their synthetic capacity for various neuroactive molecules (Martin, 1992) and their morphological plasticity rather than direct intervention in the life or death decision-making process by the injured nerve cell.

NOTE ADDED IN PROOF

Following submission of this manuscript, it was reported (Carroll et al., 1993) that primary astrocyte cultures treated with interferon-γ increased CNTF mRNA levels, as detected by Northern blot analysis. Further, (−)deprenyl has been shown to increase GFAP immunodensity as well as the relative index of astrocyte hypertrophy by using computer-assisted densitometry to assess the relative optical density of the area encompassed by reactive glia (Ju et al., 1994).

ACKNOWLEDGMENTS

This work was supported in part by a grant from the Network for Centres of Excellence, Canada (J.C.R.) and the Parkinson's Foundation of Canada (W.G.T.). N.A.S. and J.T.H. received NCE Fellowships, J.C.R. is an MRC Scientist.

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