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Developmental expression of the platelet-derived growth factor α-receptor gene in mammalian central nervous system

(*in situ* hybridization/glial cells/growth factor receptors)

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ABSTRACT **We recently reported that the platelet-derived growth factor (PDGF) A-chain gene is highly expressed in neurons of embryonic and adult mouse central nervous system and suggested that its secretion by neurons may support development and maintenance of glia. We have now analyzed the levels and sites of expression of the cognate PDGF α-receptor gene in brain and spinal cord of embryonic and adult mice by *in situ* hybridization. The predominant cell populations in both gray and white matter expressing transcripts of the PDGF α-receptor gene are glial cells or their precursors. Transcripts consistently were not detected in neurons. Expression of the PDGF α-receptor gene was first observed at embryonic day 15, increased through postnatal day 14, and fell to lower levels in adults. Expression of the α-receptor gene corresponds in temporal sequence to the developmental period of glial migration and proliferation and to the expression of PDGF A by neurons. The results indicate that glia but not neurons have the potential to respond to PDGF A and suggest that neurons influence glial cell development through paracrine regulation.**

Neuronal growth factors have been implicated as primary regulatory molecules within the nervous system (1, 2). Some growth factors that have been identified with functions outside of the nervous system appear also to influence neurons and glia, suggesting that the nervous system may be regulated by the same growth factors that function in other organs (3–5). We recently demonstrated that the platelet-derived growth factor (PDGF) A-chain gene is expressed in high levels in neuronal populations of embryonic and adult mice (5). The time course of appearance of A-chain gene transcripts and their near ubiquitous expression in neurons suggested that the neuron may be the major source of the PDGF A-chain in the nervous system and that the neuron may function in a previously undescribed role in the paracrine regulation of central nervous system (CNS) development. Earlier work had indicated that PDGF functions to regulate glial differentiation *in vitro* (6, 7). However, it was not appreciated that the neuron expressed high levels of the PDGF A-chain gene. To understand the significance of these findings, we analyzed the cellular localization and time course of expression of the gene encoding the cognate receptor for PDGF A, the PDGF α receptor (8, 9), within the CNS of embryonic and postnatal mice by *in situ* hybridization to seek these cells that coordinately express the α-receptors.

MATERIALS AND METHODS

Tissue sections including cerebral cortex, hippocampus, midbrain, thalamus, pons, cerebellum, and spinal cord from BALB/c/B10 mice at embryonic day 8 (E8), E10, E12, E15, and E18 and postnatal day 1 (P1), P7, P14, P28, and P56 were prepared as described (5).

A mouse cDNA [corresponding to residues 2198–2582 of the PDGF α receptor (9)] was made as the PCR product from mouse 3T3 cell total RNA. Preparation of ^{35}S-labeled RNA probes was described previously (5). Adjacent sections were hybridized with ^{35}S-labeled sense RNA, with excess (20×) unlabeled antisense RNA (18 hr, 55°C) before hybridization with ^{35}S-labeled antisense RNA, with excess (20×) unlabeled antisense RNA coincubated with ^{35}S-labeled antisense RNA, or digested with RNase A (50 μg/ml, 1 hr, 37°C) before hybridization with ^{35}S-labeled antisense RNA as controls to establish the specificity of labeling.

Immunostaining was described previously (5). The O_4 antibody was kindly provided by M. Dubois-Dalcq and R. D. McKinnon (National Institutes of Health). In control experiments, the antibody was replaced with 1% bovine serum albumin in phosphate-buffered saline or with normal goat serum.

RESULTS

Specifically labeled and control sections from E8, E10, E12, E15, and E18 embryos and from P1, P7, P14, P28, and P56 animals were analyzed by both darkfield and brightfield microscopy. Transcripts of the PDGF α-receptor gene were not seen in the CNS of E8, E10, or E12 embryos, whereas transcripts were readily detected in both spinal cord and brain by darkfield microscopy of E15 embryos. The ^{35}S-hybridization signal was particularly intense in developing white matter (Fig. 1 *A–C*, arrows) and found in clusters around the nuclei of small cells. The localization of the α receptor in cells within the developing white matter at E15 indicated that these were glial cells or their precursors and likely to be O-2A cells, the progenitors of the oligodendroglia in white matter. In brightfield photomicrographs of E15 brain (Fig. 1*E*), the cortical plate (asterisk) and a deeper layer that contains migrating cells (curved arrow) were identified. Transcripts were localized primarily in areas between the major cell layers (Fig. 1*D*, arrows). These results indicate that the PDGF α-receptor gene is expressed in developing white matter in both brain and spinal cord.

Transcripts of the PDGF α-receptor gene were also expressed at high levels in transverse sections of P7 spinal cord in cells that were scattered throughout the white matter and all of the laminae of spinal cord gray matter (Fig. 2*A*). Higher-power views of similar spinal cord sections are shown in Fig. 2 *B* and *C* and Fig. 4 *A–C*. Well-localized signals are seen in cells in the white matter (arrows), again establishing that glial cells or glial precursors express the PDGF α-receptor mRNA.

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Abbreviations: PDGF, platelet-derived growth factor; CNS, central nervous system; E*n*, embryonic day *n*; P*n*, postnatal day *n*.

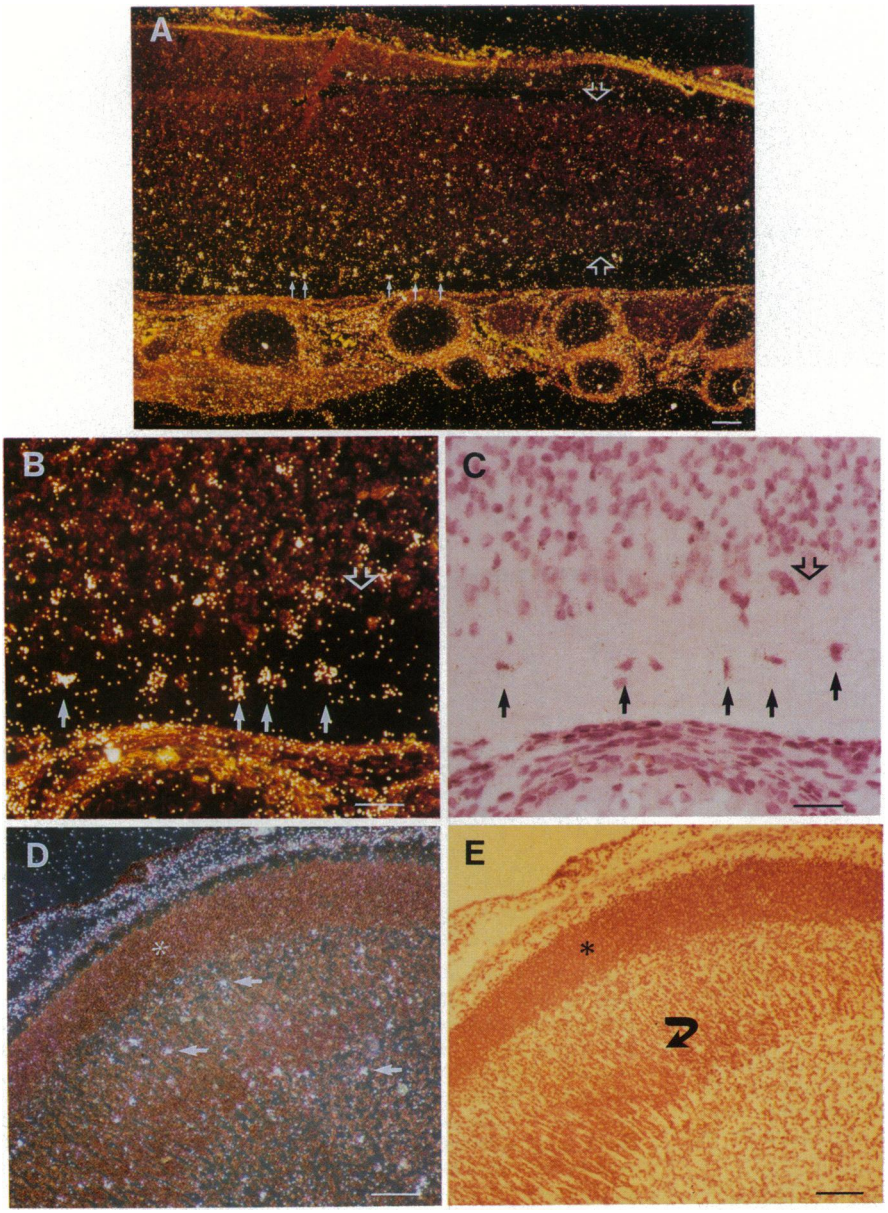

FIG. 1. (*A–C*) Sagittal sections of mouse spinal cord at E15. Open arrows indicate gray/white matter border. (*A*) Low-power darkfield view showing hybridization signal in the spinal cord (small arrows). (*B*) High-power view of *A* showing spinal gray matter, white matter, and ventral vertebral bodies. Localized clusters of grains can be seen scattered throughout both the gray and the white matter. Signal expression in white matter demonstrates that some of this expression is in glial cells or glial precursors (arrows). (*C*) Bright field of *B* showing the morphology of cells expressing PDGF α-receptor mRNA in the white matter (arrows). (*D* and *E*) Coronal sections of brain at E15 showing the cortical plate (asterisk). (*D*) Dark field showing signal expression which is outside the cortical plate and in an area populated by glial cell precursors (arrows). (*E*) Bright field of the same section as *D* to show the cortical architecture at this age. Layer of migrating cells in the intermediate zone is indicated (curved arrow). [Bar = 100 μm (*A*), 40 μm (*B*, *D*, and *E*), or 35 μm (*C*).]

Cells expressing PDGF α-receptor mRNA were also present in gray matter. In the region through lamina IX of the spinal cord, motor-neuron cell bodies were easily identified and were invariably negative (Fig. 2*C*, large arrows), whereas transcripts were seen in cells with small, darkly staining nuclei (small arrows). Motor neurons (open arrows) and spinal cord interneurons (small solid arrows) were also negative in high-power views (Fig. 4*C*), in contrast to the cells with small darkly staining nuclei (solid arrows). The cells in gray matter that expressed the PDGF α-receptor gene were not neurons and were probably glia or glial precursors (see below). The pattern of expression of the PDGF α-receptor gene in P7 mouse cortex is shown in Fig. 3*A*; the brain surface is at the top and cortical layers II and III are denoted by an asterisk. In dark field, a diffuse pattern of expression of the PDGF α-receptor gene is seen through the different regions of cortex in both gray and white matter (Fig. 3*A*). The normal neuronal architecture is seen in bright field of this same area (Fig. 3*B*). A control section hybridized with the ^{35}S-labeled PDGF α-receptor sense probe (Fig. 3*C*) established specificity. Small cells (small arrows) that expressed high levels of the α-receptor gene were interspersed with larger cells that were invariably negative; these negative cells were readily identified as neurons (large arrows) in high-power views (Fig. 3*D*). The large neuronal nuclei (open arrows) also were negative in P28 mouse cortex, whereas the small cells with darkly staining nuclei (solid arrows) showed high levels of expression (Fig. 4*G*).

To further characterize the cells that express the PDGF α receptor, we used the antibody O_4 (10, 11), which recognizes glial precursors in the brain at least until the second postnatal week (12). In the cortex (layers II and III) of a P7 mouse (Fig. 3*E*), the brown reaction product was restricted to non-neuronal elements (long arrows) and their processes (small arrows); in contrast, it was not found in neurons (thick arrows). The cells expressing the PDGF α receptor (Fig. 3*D*, small arrows) correlated directly with the cells that were O_4-positive (Fig. 3*E*), suggesting strongly that these cells were of glial lineage.

We surveyed additional areas in which neurons could be readily identified. The pyramidal cells in the hippocampus were invariably negative (Fig. 4 *E* and *F*, open arrows) whereas the small cells (see above) with darkly staining nuclei consistently expressed transcripts of the PDGF α-receptor gene (Fig. 4*E*, solid arrow). Similarly, small cells in the fimbria (Fig. 4*D*, arrows), a white matter tract containing axons of hippocampal projection neurons, in the cerebellum (Fig. 4*H*), and in the brainstem nuclei and white matter of the

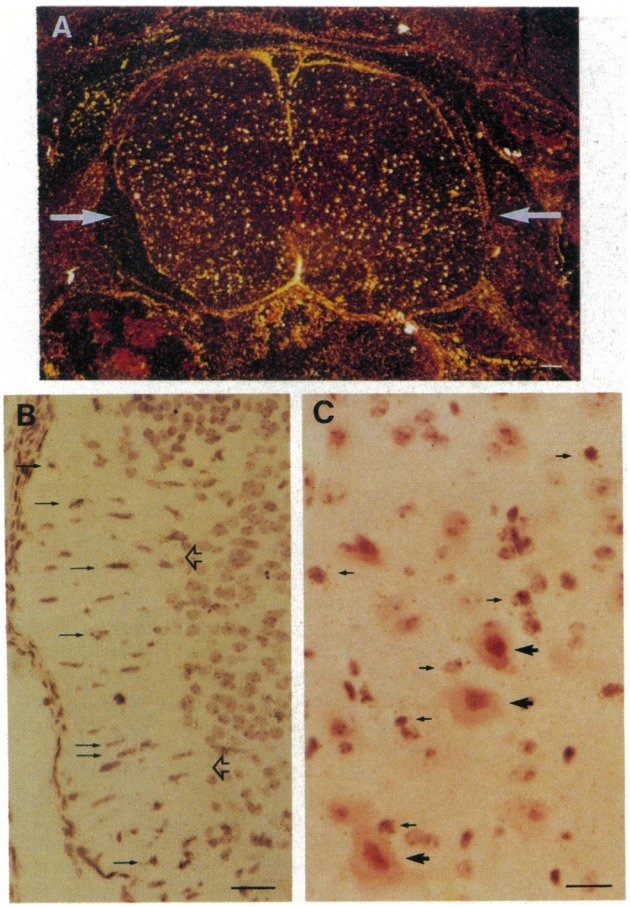

FIG. 2. Transverse sections of spinal cord from a P7 animal. (*A*) Cells expressing PDGF α-receptor mRNA are scattered throughout the cord in both gray matter and white matter. Arrows indicate border of the spinal cord. (*B*) High-power view of the lateral border of the spinal cord shows clear signal expression over glial cells in the white matter (arrows). Open arrows show gray/white matter border. (*C*) Higher-power view of the gray matter in the ventral horn shows that motor neurons (large arrows) do not express the α receptor. In contrast, some of the smaller cells with darkly staining nuclei show intense expression (small arrows). [Bar = 100 μm (*A*), 35 μm (*B*), or 30 μm (*C*).]

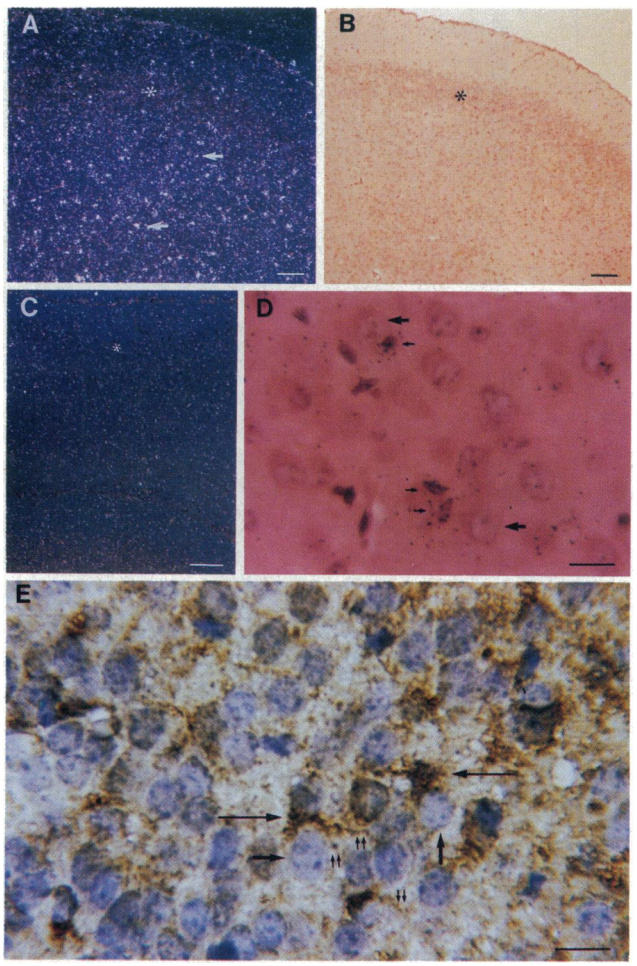

FIG. 3. Views of the cerebral cortex from a P7 animal. (*A*) Dark field at low magnification, showing that the signal is scattered throughout all the cortical layers (arrows). Asterisk, layers II and III. (*B*) Bright field of *A*, showing the normal geometry at this age. Asterisk, layers II and III. (*C*) Darkfield photomicrograph of a coronal section of cerebral cortex from a control experiment hybridized with ^{35}S-labeled PDGF α-receptor antisense RNA probe after block with nonradioactive PDGF α-receptor antisense RNA. No specific hybridization signal is present. Asterisk, layers II and III. (*D*) High-power view in gray matter of cerebral cortex, showing that large nuclei (arrows) are not surrounded by grains, but small darkly staining nuclei are (small arrows). (*E*) High-power view of cerebral cortex (layers II and III) showing immunoreactivity with O_4 antibody. O_4-positive cells (long arrows) and their processes (small arrows) are present in proximity to large cells (thick arrows) that are invariably negative for O_4 immunoreactivity. [Bar = 100 μm (*A–C*) or 15 μm (*D* and *E*).]

brainstem (data not shown) consistently were positive. The Purkinje cells (open arrows) and granule cells (asterisk) of the cerebellum (Fig. 4*H*) and the brainstem neurons in the major motor nuclei such as facial and hypoglossal nuclei (data not shown) were never observed to be positive.

We also compared the pattern of expression of the PDGF α-receptor gene with the distribution of transcripts of the PDGF A-chain gene (Fig. 5). In an E18 mouse spinal cord hybridized with an ^{35}S-labeled RNA probe complementary to the PDGF A-chain mRNA, intense hybridization signals were seen in virtually all neurons in the spinal cord and dorsal root ganglia (Fig. 5*A*, asterisks); the hybridization signal was consistently at lower levels in white matter, as previously described (5). In direct comparison, the PDGF α-receptor transcripts (Fig. 5*B*) were expressed in small cells in gray matter and were particularly prominent around the edges of the spinal cord (arrows) in developing white matter. Furthermore, the pyramidal neurons (Fig. 5*C*, asterisk) in the hippocampus of P60 mouse brain expressed the PDGF A-chain gene signal intensely, whereas expression of the PDGF α-receptor gene was not observed in the hippocampal neurons (Fig. 5*D*, asterisk). It was highly expressed in glial elements (arrows) outside the hippocampal pyramidal-cell band in the same area of the hippocampus.

DISCUSSION

This work has established that transcripts of the PDGF α-receptor gene are present in all regions of the CNS of embryonic and postnatal mice; the transcripts are first detected at E15, rise to high levels at E18, remain at high levels through the first few postnatal weeks, and decline to lower, but readily detectable, levels in adulthood. Expression is particularly intense in developing white matter that does not contain neurons; in gray matter, only cells with small, darkly staining nuclei that appear to be glial cells are positive. Also, the patterns of cells expressing the PDGF α-receptor gene are identical with those of cells recognized with the O_4 antibody, an antibody that recognizes a glycolipid determinant of oligodendrocytes and some of their progenitors, including O-2A cells (10, 11). Thus, the cells that express the PDGF α receptor are primarily glial cells or their precursors. A

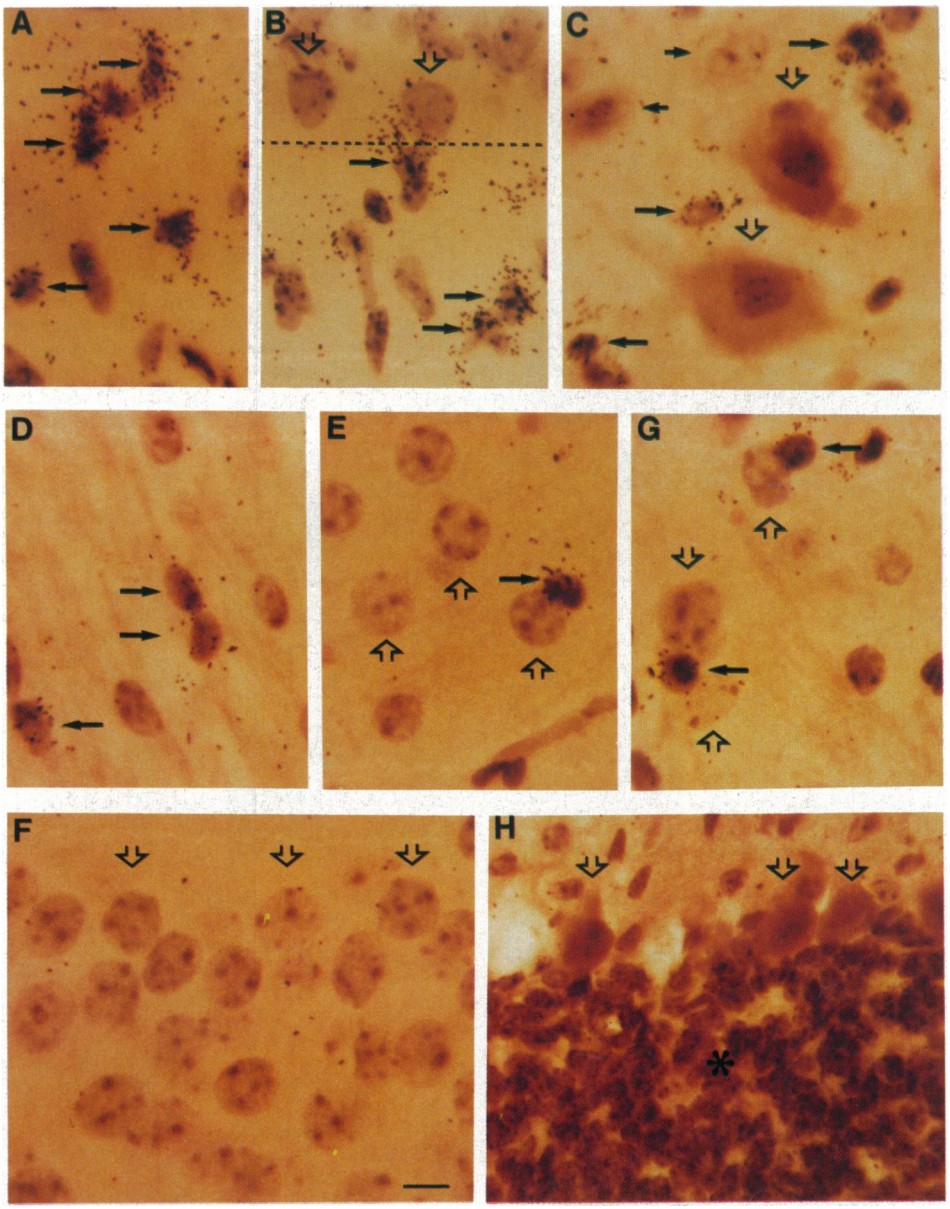

FIG. 4. High-power views of cells in the mouse CNS expressing PDGF α-receptor mRNA. (*A–C*) Transverse spinal cord sections from a P7 animal. (*A*) Section through the white matter (ventral funiculus). Darkly staining nuclei of small cells in the white matter are shown. Intense expression of PDGF α-receptor mRNA by the majority of these cells (arrows) is apparent. (*B*) Section of spinal cord taken through the transition (dashed line) between gray matter of the ventral horn and white matter of the ventral funiculus. Note intense expression of PDGF α-receptor mRNA by many of the cells in white matter (solid arrows). In contrast, no grains are associated with neurons in the gray matter (open arrows). (*C*) Section through the gray matter of the ventral horn. Large cells (open arrows) identified as motor neurons are negative for PDGF α receptor. Interneurons (small solid arrows) are also negative. In contrast, some small cells in the gray matter, presumably glia, express PDGF α-receptor message (solid arrows). (*D–F*) Sections through the hippocampus in a 1-month-old animal. (*D*) Note the expression of message by numerous cells in the fimbria (arrows). (*E*) Section through the pyramidal cell layer of the CA1 region. Large nuclei of hippocampal pyramidal cells (open arrows) are shown. None of these cells express PDGF α-receptor mRNA. In contrast, grains surround a small, darkly staining nucleus which most likely represents a glial cell. (*F*) A section through CA1 showing many nuclei of hippocampal pyramidal cells. These cells are invariably negative for PDGF α-receptor expression. (*G*) Section through layer V of cerebral cortex of a 1-month-old animal. Again the nuclei of large neurons (open arrows) are negative for PDGF α-receptor message. In contrast, grains are apparent surrounding the small, darkly staining nuclei. (*H*) Section through the cerebellum of a 1-month-old animal. This section shows cerebellar Purkinje cells with the granule-cell layer beneath. Cerebellar neurons do not express PDGF α-receptor message. (Bar = 5 μm.)

systematic survey of several regions of the CNS failed to detect expression in any neuronal population.

The ligand (PDGF A) for the PDGF α receptor is ubiquitously expressed in neurons in developing and mature mice (5). In contrast, the PDGF α receptor is not expressed by neurons but is expressed coordinately by glial cells or their precursors, suggesting that the PDGF A secreted by neurons may influence glial cells via a paracrine mechanism. *In vitro*, PDGF has been shown to influence the pattern of differentiation and the intrinsic timing of differentiation of glial precursors from rat optic nerve that lack neurons (3, 13, 14). Interestingly, in cortical cultures where neurons are present, O-2A progenitors proliferate without exogenous PDGF (14). This result suggests that the cortex replaces PDGF in support of glial differentiation and that neurons may be the primary source of PDGF. Since PDGF A is readily secreted (15, 16) it may also function as a chemoattractant for glial precursors. PDGF is known to be a potent chemoattractant for many cell types (17, 18),

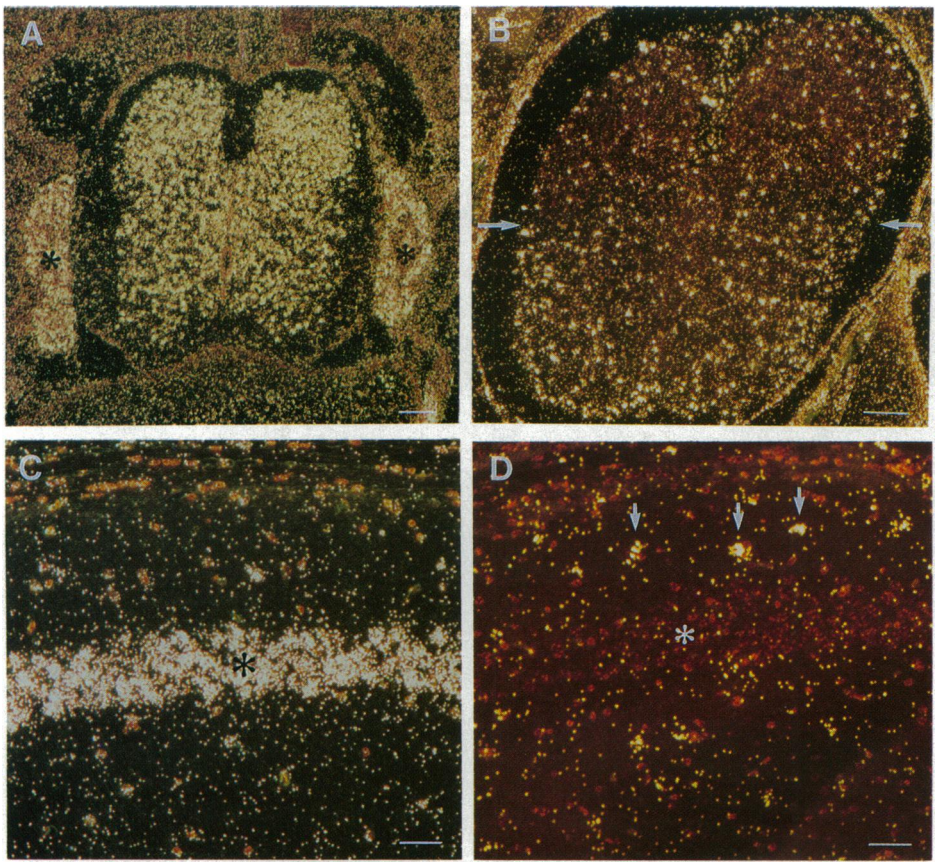

FIG. 5. Comparison of patterns of expression of PDGF A-chain mRNA and PDGF α-receptor mRNA. (*A*) PDGF A-chain mRNA in E18 spinal cord. Intense expression is shown by almost all neurons of spinal cord and dorsal root ganglia (asterisks). Expression is less intense in white matter than in gray matter. (*B*) Transverse section of E18 spinal cord hybridized with probe for the PDGF α receptor. Note scattered expression by small cells throughout the spinal cord in white matter and gray matter (arrows indicate border of the spinal cord). (*C*) Section through the hippocampal pyramidal cell layer of a 2-month-old mouse. Hybridization with probe for PDGF A chain shows intense expression by hippocampal pyramidal cells (asterisk). (*D*) Section through the same region of brain hybridized with a probe for the α receptor. Note that the pyramidal cells do not express signal (asterisk), but there is clear signal expression by cells in the white matter (arrows). [Bar = 100 μm (*A* and *B*) or 50 μm (*C* and *D*).]

including O-2A progenitor cells (19), and, *in vitro*, expression of the PDGF α receptor appears to be sufficient for cells to respond chemotactically to PDGF A (20). O-2A progenitors must leave the ventricular zone and populate characteristic locations in the adult CNS. Importantly, the expression of the PDGF α receptor observed in this work corresponds to the time that glial migration is occurring.

This work thus demonstrates that the PDGF α receptor is expressed by glial cells and that glia are competent to respond to the PDGF A secreted by neurons *in vivo*. The work also demonstrates a correspondence of expression of these two genes during late embryogenesis and in early postnatal life, supporting the idea that PDGF A regulates glial development *in vivo*. Because neurons appear to be the primary source of PDGF A in the CNS, the work supports the view that neurons may direct development of their supporting elements in the nervous system, a view consistent with earlier results indicating that other growth factors may be important in neuronal/glial cell interactions of a bidirectional nature (21, 22).

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