Hedgehog-dependent oligodendrocyte lineage specification in the telencephalon

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SUMMARY

In the caudal neural tube, oligodendrocyte progenitors (OLPs) originate in the ventral neuroepithelium under the influence of Sonic hedgehog (SHH), then migrate throughout the spinal cord and brainstem before differentiating into myelin-forming cells. We present evidence that oligodendrogenesis in the anterior neural tube follows a similar pattern. We show that OLPs in the embryonic mouse forebrain express platelet-derived growth factor alpha-receptors (PDGFRA), as they do in more caudal regions. They first appear within a region of anterior hypothalamic neuroepithelium that co-expresses mRNA encoding SHH, its receptor PTC1 (PTCH) and the transcription factors OLIG1, OLIG2 and SOX10. Pdgfra-positive progenitors later spread through the forebrain into areas where Shh is not expressed, including the cerebral cortex. Cyclopamine inhibited OLP development in cultures of mouse basal forebrain, suggesting that hedgehog (HH) signalling is obligatory for oligodendrogenesis in the ventral telencephalon. Moreover, Pdgfra-positive progenitors did not appear on schedule in the ventral forebrains of Nkx2.1 null mice, which lack the telencephalic domain of Shh expression. However, OLPs did develop in cultures of Nkx2.1−/− basal forebrain and this was blocked by cyclopamine. OLPs also developed in neocortical cultures, even though Shh transcripts could not be detected in the embryonic cortex. Here, too, the appearance of OLPs was suppressed by cyclopamine. In keeping with these findings, we detected mRNA encoding SHH and Indian hedgehog (IHH) in both Nkx2.1−/− basal forebrain cultures and neocortical cultures. Overall, the data are consistent with the idea that OLPs in the telencephalon, possibly even some of those in the cortex, develop under the influence of SHH in the ventral forebrain.

Key words: Telencephalon, Hedgehog signalling, Oligodendrocyte, Rat, Mouse

INTRODUCTION

Oligodendrocytes in the spinal cord and brainstem are derived from a subset of ventral neuroepithelial cells, under the influence of Sonic hedgehog protein (SHH) from the ventral midline (notochord and floor plate; reviewed by Miller, 1996; Rogister et al., 1999; Richardson et al., 2000; Spassky et al., 2000). SHH patterns the ventral neuroepithelium by controlling expression of a set of transcription factors including homeodomain proteins NKX2.2 and PAX6, the high mobility group (HMG) protein SOX10 and the basic helix-loop-helix (bHLH) proteins OLIG1 and OLIG2 (Ericson et al., 1997; Kuhlbrodt et al., 1998; Lu et al., 2000; Zhou et al., 2000; Takebayashi et al., 2000). The Olig genes and Sox10 are co-expressed in the oligodendrogenic part of the neuroepithelium a day or two before the appearance of Pdgfra-positive oligodendrocyte progenitors (Lu et al., 2000; Zhou et al., 2000). The Pdgfra+ progenitors then proliferate and migrate away from the ventricular surface into all parts of the spinal cord before differentiating into myelin-forming oligodendrocytes (Pringle and Richardson, 1993; Calver et al., 1998).

The origins of oligodendrocytes at more anterior levels of the neuraxis are less well established. Timsit et al. showed that the myelin proteolipid protein gene Plp/Dm20 is expressed in the ventral neuroepithelium of the embryonic mouse diencephalon from as early as E9 (Timsit et al., 1992); they proposed that this region later goes on to generate oligodendrocytes. Pringle and Richardson (Pringle and Richardson, 1993) described a cluster of Pdgfra-positive presumptive oligodendrocyte progenitors (OLPs) in the ventral forebrain of the rat embryo that appeared to proliferate and
Fig. 1. Expression of *Pdgfra*, *Shh* and *Ptc1* in the embryonic rat forebrain. (A-C), E13.5 anterior forebrain, coronal sections. *Pdgfra* is expressed in the neuroepithelium and adjacent SVZ at the boundary between anterior hypothalamus and MGE (arrowhead in A) within broader domains of *Shh* (B) and *Ptc1* (C) expression. There is an additional domain of *Shh* and *Ptc1* expression in the preoptic recess (arrow in B), but there is no *Pdgfra* expression in this region. (D) Coronal and (E) parasagittal sections of E14.5 anterior forebrain. *Pdgfra* is widely expressed outside the nervous system (D). In the ventral forebrain, *Pdgfra* is strongly expressed in the VZ and SVZ of the anterior hypothalamus, extending dorsally into the MGE (D), but is not expressed in the LGE or cortex at this stage. Strong expression is also observed in the primordia of the choroid plexus (arrow in D). In the SVZ, two categories of *Pdgfra*+ cells are intermingled: closely packed cells that express relatively low levels of *Pdgfra* and smaller cells that are more intensely labelled (arrowheads in E). (F) Diagram to show the plane of section of A-D and the location of the field shown in E. MGE and LGE, medial and lateral ganglionic eminences; Cx, cerebral cortex; Tel, telencephalon; Di, diencephalon; 3V, third ventricle.

Fig. 2. Scattered *Pdgfra*+ cells spread into the cerebral cortex in lateral-to-medial and ventral-to-dorsal directions. (A-C) Coronal sections through the neocortex at different ages, hybrized in situ with a probe for *Pdgfra*. (A) At E14.5, there are no *Pdgfra*+ cells in the developing cortex. (B) By E17.5, intensely labelled *Pdgfra*+ cells are present within the developing cortical plate and presumptive sub-cortical white matter at the lateral margin of the cortex as well as the medial cortex (arrowheads in B). (C) By E20.5, there are numerous *Pdgfra*+ cells in the cortex, especially in the sub-cortical white matter. At all stages, *Pdgfra* is also expressed by the meninges and skull.

Fig. 3. Overlapping expression of *Pdgfra*, *Sox10*, *Olig1*, *Olig2* and *Shh* in the ventral forebrain and neocortex. (A-E) Serial coronal sections of rat E14.5 forebrain. The four presumptive oligodendrocyte lineage markers show overlapping but non-identical patterns of expression in the neuroepithelium, SVZ and mantle zones of the anterior hypothalamus and MGE. Their expression domains also overlap *Shh* expression in the neuroepithelium (E). The neuroepithelial expression of *Olig2* extends further than the rest, through the MGE and LGE (D). (F) Diagram to indicate the approximate position of sections A-E. (G-K) Serial coronal sections through the rat cortex at E18.5. Cells expressing *Pdgfra*, *Sox10*, *Olig1* and *Olig2* have a similar scattered distribution in the cortex at this stage. *Shh* cannot be detected in the cortex (K). The position of sections G-K is indicated in L. Cx, cortex; Th, thalamus; Hy, hypothalamus; BG, basal ganglia.
HH-dependent oligodendrogenesis in the forebrain

migrate throughout the developing forebrain. Spassky et al. and Perez-Villegas et al. also provided histological evidence for a ventral source of oligodendrocytes in the rodent and chick forebrains, respectively (Spassky et al., 1998; Perez-Villegas et al., 1999). In keeping with all these studies, it has been reported that precursor cells from E15 rat striatum (ventral telencephalon) have a greater propensity to generate oligodendrocytes than do precursors from the neocortex (dorsal telencephalon), either when cultured in vitro (Birling and Price, 1998) or when transplanted into the eye (Kalman and Tuba, 1998).

It therefore seems likely that there is a region of the ventral forebrain that is specialized for oligodendrogenesis. The experiments reported here further define the location of this site and explore how and when it is established. Our data support the idea that oligodendrogenesis in the rodent telencephalon depends on a localized source of SHH in the ventral forebrain. Even in cultures derived from embryonic neocortex, which does not appear to express SHH or related molecules in situ, generation of oligodendrocyte progenitors was blocked by cyclopamine and appeared to depend on SHH and/or IHH produced in the cultures. Altogether, the available data suggest that at least some oligodendrocytes in the cortex might be derived from progenitor cells that originate in the basal telencephalon – as, for example, the precursors of certain cortical neurons (Parnavelas, 2000).

MATERIALS AND METHODS

Animals

Wild-type rats (Sprague-Dawley) and mice (C57BL/6J) were bred in-house at University College London (UCL). Nkx2.1 (Titf1) knockout...
mice were maintained on the 129/Sv or Black/Swiss hybrid background. Midday following appearance of the vaginal plug was designated embryonic day 0.5 (E0.5). Hence, our ages are 0.5 days younger than those of Altman and Bayer (Altman and Bayer, 1995) (e.g. our E13.5 corresponds to their E14). Nkx2.1 knockout mice (Kimura et al., 1996) and their wild-type littermates were genotyped by polymerase chain reaction (PCR). Primers for amplifying wild-type and mutant alleles were 5'-TCT TGT AGC GGT GCT TCT GGA-3' and either 5'-GGC GAG CCG CAT GAA TAT GA-3' (wild-type allele, approx. 250 bp product) or 5'-TCC CCT TCT ATC GCC TTC TTG AGG AG-3' (null allele, approx. 220 bp product).

**Tissue preparation and in situ hybridization**

The heads of embryos were fixed by immersion in 4% (w/v) paraformaldehyde (PFA) in phosphate-buffered saline (PBS) pH7.5 for 24 hours at 4°C. To aid fixation, the skin and skull were removed from E14.5 and older embryos. Tissue was cryoprotected in 20% (w/v) sucrose in PBS and embedded in OCT (Raymond Lamb). The heads of embryos were fixed by immersion in 4% (w/v) paraformaldehyde (PFA) in phosphate-buffered saline (PBS) pH7.5 for 24 hours at 4°C. To aid fixation, the skin and skull were removed from E14.5 and older embryos. Tissue was cryoprotected in 20% (w/v) sucrose in PBS and embedded in OCT (Raymond Lamb).

**Tissue preparation and in situ hybridization**

**Immunoselection**

A single-cell suspension of E19 rat brain (minus brainstem), neocortex or striatum, prepared as described above, was passed over two bacteriological Petri dishes coated with monoclonal antibody Ran-2 (Bartlett et al., 1981) to remove astrocytes, meningeal cells and macrophages (the latter by non-specific adherence to plastic), then over a dish coated with anti-PDGFRα rabbit serum (#3979; Fretto et al., 1993) as described by Hall et al. (Hall et al., 1996). The PDGFRα-positive (PDGFRA+) cells were removed from the panning dish with trypsin and plated (1000 cells in a 3 µl droplet) on 6 mm diameter poly-D-lysine-coated glass coverslips in defined medium (Bottenstein and Sato, 1979) containing 0.5% or 10% FCS.

**Immunocytochemistry**

Cells on coverslips were lightly fixed in 4% (w/v) PFA in PBS for 5 minutes at room temperature and washed in PBS. The following primary antibodies were used: anti-PDGFRα rabbit serum, (#3979; Fretto et al., 1993) diluted 1:100 in PBS, monoclonal antibody A2B5 (Eisenbarth et al., 1979), anti-NG2 rabbit serum (Chemicon) or monoclonal anti-NG2 (clone N11.4; Stallcup and Beasley, 1987; Levine and Stallcup, 1987), monoclonal antibody O4 (Sommer and Schachner, 1981; Sommer and Schachner, 1982; Bansal et al., 1992), monoclonal anti-galactocerebroside (GC; Ranscht et al., 1982; Bansal and Pfeiffer, 1992) monoclonal anti-glial fibrillary acidic protein (GFAP; clone GA-5, Sigma). For intracellular antigens the cells were made permeable with 0.1% (v/v) Triton X-100 in PBS. Primary antibody treatments were for 1 hour in a humid chamber at room temperature. Fluorescent secondary antibodies (Perbio Science, UK) were applied for 30 minutes at room temperature. Cells were post-fixed for 5 minutes in 4% (w/v) PFA in PBS and mounted under coverslips in Citifluor (City University, UK).

**RT-PCR**

RNA was prepared from freshly dissociated or cultured cells using Trizol® reagent (Gibco BRL). Reverse-transcription was carried out using the Superscript™ first-strand synthesis system (Gibco BRL). Primers for polymerase chain amplification (PCR) of mouse or rat cDNAs were as follows: Shh, 5′-GGG ATG ATG AAC CAG TGG CCT GG3′ and 5′-GCC GCC ACG GAG TTC TCT GC-3′; Ihh, 5′-GGG CAT CTC TGT CAT GAA CC-3′ and 5′-CAG CCA CCT GCT TGG GCA GC-3′; Dhh, 5′-GGT CGG CAG CAA CAA CTT GTG CC-3′ and 5′-GAA TCC TGT GCC TGG TGC CC-3′. Gapdh, 5′-CCC AGA ACA TCA TCT CTC G-3′ and 5′-GCC ATG AGG TCC ACC ACC C-3′. Typically, we employed a 38 cycle PCR reaction, except for Gapdh where the number of cycles was reduced to 30 to avoid saturation.

**RESULTS**

**Overlapping expression of Pdgfra, Shh and Ptc1 in the ventral forebrain**

In the E13.5 rat forebrain, Pdgfra was expressed in the ventricular and subventricular zones (VZ and SVZ) in a restricted region spanning the boundary between the anterior hypothalamus (diencephalon) and the medial ganglionic eminence (MGE; telencephalon) (Fig. 1A; Altman and Bayer, 1995). This region has been called the anterior entopeduncular area (AEP; Puelles et al., 2000). The focus of Pdgfra lay within broader expression domains of Shh and Ptc1 (Fig. 1B,C). At E14.5, there was a large number of faintly labelled, close-packed Pdgfra+ cells within the SVZ (Fig. 1D,E). Among and
null cultures did not develop OLPs until 4-6DIV. Nkx2.1, Gapdh, Shh, 382 bp, Ihh, 267 bp; Dhh, Shh, 311 bp; Dhh
markers (M) are 200 bp and 400 bp. The sizes of PCR products were be detected by RT-PCR throughout the culture period. Molecular size were detected. (B) Transcripts for SHH and IHH but not DHH could inhibited by cyclopamine. Asterisks denote that no NG2 + progenitors
The development of OLPs in ventral forebrain cultures of wild-type and Nkx2.1-/- mice. Dissociated ventral forebrain cells from E13.5 wild-type and Nkx2.1-/- mice were cultured in defined medium for up to 8DIV in the presence or absence of the HH inhibitor cyclopamine (cyc, 1 µM). The cultures were immunolabelled with polyclonal anti-NG2 to visualize OLPs and the average number of NG2+ cells per 20 fields (>63 microscope objective) was determined for different times in culture. (A) Wild-type cultures contained NG2+ process-bearing cells from the outset, whereas Nkx2.1 null cultures did not develop OLPs until 4-6DIV. The development of OLPs in Nkx2.1-/- cultures was strongly inhibited by cyclopamine. Asterisks denote that no NG2+ progenitors were detected. (B) Transcripts for SHH and IHH but not DHH could be detected by RT-PCR throughout the culture period. Molecular size markers (M) are 200 bp and 400 bp. The sizes of PCR products were as predicted: Shh, 242 bp; Ihh, 267 bp; Dhh, 311 bp; Gapdh, 382 bp.

around these were scattered some smaller, more intensely labelled cells (Fig. 1E, arrowheads). After E14.5, the intensely labelled cells became more numerous and widely distributed, reaching into the SVZ and mantle zones of the MGE and the lateral ganglionic eminence (LGE), while the close-packed Pdgfra+ cells in the SVZ became less numerous and disappeared around E15.5. By E17 there were small Pdgfra+ cells scattered more-or-less uniformly throughout the ventral forebrain (not shown). We purified these by immunoselection and showed that they generate oligodendrocytes in vitro (see below).

Lateral-to-medial spread of Pdgfra+ cells in the cortex suggests immigration from the basal forebrain

Pdgfra+ cells began to appear at the cortico-striatal boundary near the lateral tips of the lateral ventricles around E16.5. By E17.5, some were present within the cortical plate and presumptive sub-cortical white matter (Fig. 2B). By E20.5 (just before birth) many Pdgfra+ cells were present throughout the cortical plate, though they were concentrated in the subcortical white matter (Fig. 2C). They accumulated mainly in a lateral-to-medial direction from the tips of the lateral ventricles but they also appeared to enter the medial cortex in a ventral-to-dorsal direction (Fig. 2B, arrowheads). Pdgfra was not detected in the cortical VZ at these ages.

One possible interpretation of these data is that the faint Pdgfra+ cells in the anterior hypothalamic neuroepithelium give rise to intensely labelled Pdgfra+ cells, which then migrate away to populate the entire telencephalon. However, we have no direct evidence for migration.

Expression of Sox10, Olig1 and Olig2 in the telencephalon

Recent studies have shown that two novel SHH-inducible bHLH genes, Olig1 and Olig2, as well as the HMG protein Sox10, are expressed in oligodendrocyte lineage cells in the spinal cord, including the oligodendrogenic part of the ventral VZ where Pdgfra+ progenitors originate (Kuhlbrodt et al., 1998; Lu et al., 2000; Zhou et al., 2000; Takebayashi et al., 2000). We therefore compared expression of Sox10, the Olig genes and Pdgfra in the rat telencephalon. At E14.5 all of these were expressed in the anterior hypothalamic neuroepithelium and in scattered cells in the adjacent SVZ and mantle zone (Fig. 3A-F). Olig2 was more widespread in the neuroepithelium than the others, extending through the MGE and LGE.

In the E18.5 cerebral cortex, the expression patterns of Pdgfra, Sox10, Olig1 and/or Olig2 were very similar (Fig. 3G-L). They were all expressed by cells in a scattered distribution from the lateral edges of the cortex towards the midline. We cannot tell whether these cells in serial sections represent one and the same population of cells that express all four genes, or overlapping populations that express different subsets of genes. It is possible, nevertheless, that they represent OLPs in the course of migrating into the cortical plate from the ventral telencephalon. Note that we could not detect Shh expression anywhere in the cortex at E18.5 (Fig. 3K) or indeed at any other age between E12.5 and P2 (Fig. 1 and not shown).

PDGFRα+ cells in the embryonic rat brain are oligodendrocyte progenitors

To test directly whether PDGFRα+ cells in the telencephalon...
are OLPs, we purified them from E19 rat brains (minus 
brainstem), neocortex or striatum by immunoselection with 
antibodies raised in rabbits against PDGFRA (Hall et al., 1996; 
A. C. Hall, PhD thesis, University of London, 1999; see 
Materials and Methods). After overnight culture in defined 
medium containing 0.5% FCS and 10 ng/ml PDGF-AA, which 
stimulates progenitor cell proliferation and inhibits 
differentiation (Raff et al., 1988; Richardson et al., 1988; Noble 
et al., 1988), more than 99% of the immunoselected cells had 
the morphological and antigenic features of early OLPs: i.e. 
they were small process-bearing cells that labelled with anti-
O4 or anti-GC, which identify later stages of the 
oligodendrocyte lineage, nor with anti-glial fibrillary acidic 
protein (anti-GFAP; not shown). They did not label with 
PDGFRA is co-expressed with markers for early stages of the 
oligodendrocyte lineage, nor with anti-glial fibrillary acidic 
protein (anti-GFAP; not shown). After overnight culture in the 
presence of PDGF, the medium was replaced with defined 
medium lacking PDGF and containing either 0.5% or 10% 
FCS. After a further 36 hours in the presence of 0.5% FCS, the 
immunoselected cells developed a more highly branched 
morphology and many were O4+ (not shown), typical of late 
oligodendrocytes (Fig. 4B). In the presence of 10% FCS, most 
of the immunoselected cells differentiated into GFAP+ 
astrocytes (Fig. 4C), many of which also labelled with A2B5 
(Fig. 4D). Therefore, the great majority of PDGFRA+ cells in 
the late embryonic forebrain, including the neocortex, 
resemble the oligodendrocyte-type-2 astrocyte (O-2A) 
progenitors previously characterized in cultures of cells from 
perinatal rat optic nerve (Raff et al., 1983) or spinal cord (Hall 
et al., 1996). We refer to these as OLPs. These data are in 
accord with previous observations in situ showing that 
PDGFRA is co-expressed with markers for early stages of the 
oligodendrocyte lineage in several regions of the brain 
including the cerebral cortex (Ellison and de Vellis, 1994; 
Nishiyama et al., 1996).

HH signalling is required for oligodendrogenesis in 
the ventral forebrain

In view of the described parallels between oligodendrogenesis 
in the spinal cord and forebrain, we asked whether Sonic 
hedgehog (SHH), which is obligatory for oligodendrocyte 
lineage specification in the cord, might also be required in the 
forebrain.

We cultured E10.5 mouse ventral forebrain cells in the 
presence or absence of the drug cyclopamine, which inhibits 
HH signalling (Cooper et al., 1998; Incardona et al., 1998; 
Taipale et al., 2000). The cells were then fixed and OLPs 
visualized with anti-NG2 antibodies. In the absence of 
cyclopamine, significant numbers of NG2+ process-bearing 
progenitor cells appeared between 4 and 6 days in vitro (4-
6DIV; Fig. 5). Cyclopamine strongly inhibited the appearance 
of these cells (Fig. 5), suggesting that in the forebrain, as in 
more posterior regions of the CNS, oligodendrogenesis is 
under the control of SHH and/or other related hedgehog 
molecules.

To further assess the role of SHH in vivo we examined mice 
with a targeted deletion in the Nkx2.1 homeobox gene. In these 
mice, the most anterior region of Shh expression (in the basal 
forebrain) is absent or greatly reduced, while more posterior 
regions of Shh expression remain intact (Fig. 6B.D) (Sussel 
et al., 1999). At E12.5 numerous OLPs expressing Pdgfra, Sox10, 
Olig1 and Olig2 were scattered in the anterior hypothalamus 
and MGE of wild-type mice but were undetectable in Nkx2.1 
mutant littermates (Fig. 6A,C and data not shown). This

Table 1. Development of NG2+ OLPs in forebrain explant 
cultures

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<th>Age</th>
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Fig. 9. Hedgehog-dependent 
development of OLPs in neocortical 
cultures. (A) Dissociated cells from 
E15.5 wild-type rat neocortex were 
cultured in defined medium for up to 
8DIV in the presence or absence of 
cyclopamine (cyc, 1 μM). The 
cultures were immunolabelled with 
polyclonal anti-NG2 to visualize 
OLPs and the number of NG2+ cells 
per 18 randomly chosen fields (×63 
microscope objective) was 
determined after different times in 
culture. OLPs did not develop in E15.5 
rat cortical cultures until 4-6DIV (see 
Fig. 8), but after that the number of 
OLPs increased, peaking after 
7DIV. (B) Production of OLPs was inhibited strongly by cyclopamine (A) in a dose-dependent manner. (C) Transcripts encoding SHH and IHH 
could be detected by RT-PCR as soon as 1 hour after cell dissociation (lane 0), and throughout the culture period up to 8DIV. mRNA coding for 
DHH was also detected weakly after dissociation and after 8DIV but not at intermediate times. Sizes of PCR products were as predicted (see 
Fig. 7).
situation persisted until E14.5. At E16.5, however, a sparse population of Pdgfra+ cells appeared in the telencephalon and by E18.5 there were almost normal numbers of OLPs throughout the cortex and basal ganglia (not shown). We do not know where these cells originate but their scattered distribution outside of the VZ and their delayed appearance raise the possibility that Pdgfra+ cells migrate into the telencephalon of Nkx2.1 null mice from more posterior sites of oligodendrogenesis in the diencephalon or beyond. It is known that OLPs in other regions of the CNS can migrate significant distances during normal embryonic development (Small et al., 1987; Miller et al., 1997; Ono et al., 1997; Pringle et al., 1998).

To avoid the possibility of Pdgfra+ progenitors migrating into the Nkx2.1 null telencephalon from the diencephalon, we dissected basal ganglia from E13.5 Nkx2.1 null mice – before any Pdgfra+ cells could be detected in situ – then dissociated the cells and cultured them in vitro. Unexpectedly, NG2+ progenitors did develop in these cultures; however their appearance was delayed by 4-6DIV relative to wild-type cultures (Fig. 7A). Nevertheless, by 6DIV there were similar numbers of NG2+ progenitors in wild-type and mutant cultures. Appearance of these cells in both wild-type and Nkx2.1 null cultures was strongly inhibited by cyclopamine (Fig. 7A), so we tested for the presence of transcripts encoding SHH, Indian hedgehog (IHH) and Desert hedgehog (DHH) in the starting cell populations and after culturing for up to 8DIV. We detected Shh and Ihh in wild-type (not shown) and Nkx2.1–/– basal ganglia within one or two hours of dissociating the cells and throughout the culture period of 2-8DIV (Fig. 7B). Dhh was detected only in freshly dissociated cells (Fig. 7B). It appears likely that the appearance of NG2+ progenitors in vitro is related to the presence of cells expressing SHH and/or IHH in the cultures.

Latent oligodendrogenic potential of cortical precursors

HH signalling seems to be required for oligodendrogenesis in basal forebrain cultures (see above). Since we were never able to observe expression of Shh, Ihh or Dhh in the embryonic cortex by in situ hybridization (Figs 1B, 3K and data not shown), this raised the question of whether oligodendrocytes ever normally develop in vivo from indigenous cortical precursors. As already described, the way Pdgfra+ progenitors first appear in the ventral forebrain and spread dorsally suggests that OLPs might invade the cortex from germinal zones in the ventral forebrain. We investigated the oligodendrogenic potential of indigenous cortical precursors more directly by culturing explants from E15.5 and E18.5 rat cortex (before and after the apparent influx of Pdgfra+ progenitor cells) in defined basal medium. We then fixed and labelled the cells with anti-PDGFRα or anti-NG2 to visualize OLPs or with anti-NC to visualize differentiated oligodendrocytes.

Control explants of E15.5 rat MGE (Fig. 8C, D) or E18.5 neocortex (not shown) contained numerous PDGFRα+ NG2+ cells after overnight incubation and they increased in number during the culture period (Fig. 8D; Table 1 and not shown). GC+ oligodendrocytes also developed in these cultures after 8DIV (not shown). In contrast, no PDGFRα+ or NG2+ progenitor cells were generated from E15.5 cortical explants either overnight or after 4DIV (Fig. 8A; Table 1). However, if E15.5 cortical cells were cultured for a longer period of time – 6DIV or more – many PDGFRα+ NG2+ OLPs did then appear (Fig. 8B and Table 1). Adding cyclopamine to E15.5 cultures strongly inhibited production of OLPs in a dose-dependent manner (Fig. 9A,B), indicating that in long-term cortical cultures SHH or a related molecule (IHH or DHH) was responsible for OLP development. This conclusion was supported by the fact that we could detect mRNA encoding SHH and IHH in cultured cells from E15.5 cortex (Fig. 9C).

DISCUSSION

Oligodendrogenesis in the telencephalon is a ventral, SHH-dependent process

In the spinal cord and brainstem, oligodendrocytes develop from migratory Pdgfra+ progenitor cells that are generated in the ventral neuroepithelium under the influence of SHH from the ventral midline (Pringle et al., 1996; Poncet et al., 1996; Orentas and Miller, 1996; Orentas et al., 1999). The appearance of these progenitors is prefigured by expression of the transcription factors Olig1, Olig2 and Sox10 in the ventral neuroepithelium (Kuhlbrodt et al., 1998; Lu et al., 2000; Zhou et al., 2000; Takebayashi et al., 2000). The Pdgfra+ progenitors subsequently proliferate and migrate throughout the spinal cord and brainstem. Our data reveal that oligodendrogenesis in the forebrain is similar to this in several respects.

(1) Specification of OLPs in the forebrain, as in the spinal cord, is dependent on HH expression and activity. (2) Expression of Sox10, Olig1 and Olig2 overlaps with that of Pdgfra in the ventral telencephalon, as in the ventral spinal cord. (3) Pdgfra+ OLPs spread laterally and dorsally from their site of first appearance in the ventral telencephalon, just as OLPs spread from their site of origin in the ventral spinal cord. These analogies suggest a common molecular mechanism of oligodendrocyte specification in the telencephalon and more caudal regions of the CNS. Our suggestion that Pdgfra+ OLPs in the embryonic cortex arrive there by physical migration from the ventral forebrain is at present a hypothesis that will require direct experimental verification.

Oligodendrogenesis in the ventral telencephalon appears to be dependent on locally produced SHH from ventral neuroepithelial cells. The neuroepithelial expression domain of Pdgfra in the anterior hypothalamus is nested within broader expression domains of Shh and its receptor Ptc1, indicating that the forerunners of Pdgfra+ progenitors are normally exposed to and responsive to SHH. Oligodendrogenesis in cultures of rat ventral forebrain cells was blocked by cyclopamine, an inhibitor of HH signalling pathways. Moreover, Pdgfra+ OLPs did not develop in the VZ or SVZ of the ventral forebrain of Nkx2.1 null mutant mice, which lack the telencephalic domain of Shh expression. Further evidence for the involvement of SHH comes from in vivo gain-of-function experiments in which SHH was ectopically expressed in the telencephalic neuroepithelium of mouse embryos using a retrovirus vector (Nery et al., 2001). SHH-expressing cells developed into OLPs and later into oligodendrocytes (Nery et al., 2001). These cells would not normally express SHH persistently but, nevertheless, this experiment provides a striking demonstration of the OLP-inducing activity of SHH in vivo.
Pdgfra<sup>+</sup> progenitors did appear belatedly in the telencephalon of Nkx2.1 null mice. However, they first appeared in small numbers scattered outside the germinal zones, not tightly packed within the VZ and SVZ as in wild-type mice, suggesting that Pdgfra<sup>+</sup> cells might migrate into the Nkx2.1 null forebrain from more caudal regions where Shh expression is unaffected in the mutant. OLPs also appeared in cultures derived from Nkx2.1 null ventral forebrain, where there was no possibility of immigration from other brain regions. However, their production was suppressed by cycloamine and furthermore we could detect expression of Shh and Ihh in the cultures, so HH signalling was presumably responsible for OLP induction in vitro. Whether the Shh and Ihh expression we detected reflects aberrant up-regulation of these molecules following cell dissociation, or normal expression that is undetectable in situ, we do not know. In any case, it is not necessary to invoke a SHH-independent pathway of oligodendrogenesis to explain the appearance of OLPs in the Nkx2.1 null telencephalon, as suggested recently by Nery et al. (Nery et al., 2001). Indeed, expression of HH in culture might explain the observation that some OLPs developed in cultures derived from Shh knockout brain (Nery et al., 2001). This could be tested by culturing Shh null brain cells in the presence of cycloamine. It remains possible that there is a SHH-independent (or HH-independent) route(s) to oligodendrocyte development but this requires further investigation.

Do cortical oligodendrocytes originate in the ventral telencephalon?

A possibility raised by this work is that some cortical oligodendrocytes might develop, not from endogenous cortical precursors, but rather from OLPs that migrate from the ventral forebrain. At present we have no direct evidence for migration. Nevertheless, Pdgfra<sup>+</sup> OLPs in the telencephalon appear similar to those in optic nerve or spinal cord, which are known to migrate relatively long distances during development (Small et al., 1987; Miller et al., 1997; Ono et al., 1997; Pringle et al., 1998) or following transplantation into dysmyelinating or demyelinating hosts (e.g. Warrington et al., 1993; Vignais et al., 1993). Moreover, there is a delay in appearance of Pdgfra<sup>+</sup> progenitors in the cortex of Nkx2.1 mutant embryos, which are primarily defective in ventral structures (Sussel et al., 1999), consistent with the idea that at least the early-appearing cortical OLPs are derived from the ventral forebrain. There is ample precedent for migration of progenitor cells into the neocortex from ventral telencephalon. For example, the progenitors of many GABAergic non-pyramidal neurons migrate into the developing cortex from the MGE or LGE (Anderson et al., 1997; Lavdas et al., 1999; reviewed by Parnavelas, 2000).

Latent oligodendrogenic potential of cortical precursors

We found that E15.5 rat cortical cells did not generate oligodendrocyte lineage cells in short term (4DIV) cultures, in contrast to cells from E15.5 ventral forebrain or E18.5 cortex. This confirms the finding of Birling and Price (Birling and Price, 1998) that E15 rat cortical cells have a reduced oligodendrogenic capacity in vitro compared to either E15 striatal cells or E18 cortical cells. It also tallies with the experiments of Kalman and Tuba (Kalman and Tuba, 1998) who showed that fragments of E18 rat cortex, but not E15 cortex, generate oligodendrocytes when transplanted into the eye of a new-born rat. Thus, the oligodendrogenic capacity of rat neocortex increases markedly between around E15 and E18, consistent with and as predicted by the in situ hybridization data, which show Pdgfra<sup>+</sup> progenitors apparently migrating into the cortex after E16.5 (Figs 3, 4).

Nevertheless, E15.5 rat cortical cells did generate OLPs when cultured for long periods of time (26DIV), demonstrating that rat cortical precursors have latent oligodendrogenic potential. This is analogous to the recent report that E14 rat dorsal spinal cord cells can generate oligodendrocytes in long-term, though not short-term cultures (Sussman et al., 2000). Several previous studies have demonstrated that early cortical cells from rodents can generate oligodendrocytes in vitro (e.g. Williams et al., 1991; Davis and Temple, 1994).

The appearance of OLPs in long-term cultures of E15.5 rat neocortex was puzzling, since we could not detect expression of Shh, Ihh or Dhh in the embryonic cortex by in situ hybridization. However, production of oligodendrocytes in vitro was inhibited by cycloamine and, in addition, mRNAs coding for SHH and IHH could be detected in the cultures. Perhaps HH expression is normally repressed in the cortex in vivo but under our culture conditions the inhibitory signals are rendered ineffective by dilution or otherwise. Note that Shh is clearly expressed in certain neurons in the rat cortex from about a week after birth (Traiffort et al., 1999 and our unpublished results). Whether this late-onset expression contributes to postnatal oligodendrogenesis is not known.

Is Olig2 oligodendrocyte lineage-specific?

We found that Olig2 is expressed more widely than Olig1, Sox10 or Pdgfra in both the neuroepithelium and the surrounding mantle zone (not shown). Whether the Olig<sup>−</sup>Olig<sup>+</sup> cells are OLPs is an open question, since it is by no means clear that the Olig genes and particularly Olig2 are restricted to the oligodendrocyte lineage(s). It is known, for example, that Olig2 is expressed by olfactory neurons and their precursors in the olfactory epithelium (Takebayashi et al., 2000). It is possible that the Olig<sup>−</sup>Olig<sup>+</sup> cells in the telencephalon might be related to the pluripotent neuroglial precursors that Goldman and colleagues identified in the germinal zones surrounding the lateral ventricles after birth (Levison and Goldman, 1993; Levison and Goldman, 1997).

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