Platelet-derived growth factor is constitutively secreted from neuronal cell bodies but not from axons

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Neurons synthesise and secrete many growth and

Figure 2

Hyperplasia of retinal astrocytes indicates the presence of transgenic mouse *GFAP* promoter (*GFAP–hPDGF-A*; Figure 1). In *NSE–hPDGF-A* mice, *hPDGF-A* was expressed by RGCs and cells in the inner nuclear layer of the retina (Figure 2b and [4]), and in *GFAP–hPDGF-A* mice the transgene was expressed in retinal astrocytes (Figure 2c). The effects of the transgene in the retina were analysed by visualising retinal astrocytes by *in situ* hybridisation with a probe against *PDGFRα*. In all transgenic mouse strains, there was marked hyperplasia of retinal astrocytes (Figure 2f–h). In *NSE–hPDGF-A* and *NSE–mPDGF-A* mice, this reflected PDGF-AA release from RGC cell bodies, resulting in a paracrine neuron–astrocyte interaction in the retina. This confirms that the transgene is secreted in an active form by RGCs. In *GFAP–hPDGF-A* mice, a PDGF-AA–PDGFRα autocrine loop is created in retinal astrocytes themselves.

We confirmed by *in situ* hybridisation that in *NSE–hPDGF-A* P1 mice there was no production of transgenic *PDGF-A* mRNA in the optic nerve (Figure 3b). This was as expected, as there are no resident neurons in the nerve. In contrast, we found strong transgene expression by optic nerve astrocytes in *GFAP–hPDGF-A* mice (Figure 3c), again as expected. At P1, proliferating OLPs are just starting to populate the optic nerve by inward migration from the brain through the optic chiasm [1]. Therefore, we analysed OLP numbers in the optic nerve at P7, by which time the nerve is normally full of OLPs [1]. We visualised OLPs in the optic nerve by *in situ* hybridisation for *PDGFRα*. No increase of OLP numbers could be detected in the optic nerves of either *NSE–hPDGF-A* or *NSE–mPDGF-A* mice (Figure 3f,h), suggesting that no extra PDGF is released into the optic nerves of these mice. In contrast, we found a dramatic increase of *PDGFRα*$^+$ OLP numbers in the optic nerves of *GFAP–hPDGF-A* mice compared with wild-type littermates (Figure 3g). Counting OLPs in optic nerve cross sections revealed no differences between wild-type (average 25 ± 2), *NSE–hPDGF-A* (24 ± 2) or *NSE–mPDGF-A* (24 ± 3) mice but about a threefold increase in *GFAP–hPDGF-A* mice (77 ± 8; see Materials and methods). This was accompanied by a marked increase in the diameter of the optic nerve (Figure 3g). Closer inspection by electron microscopy revealed that this size increase resulted from an increase of oligodendrocyte lineage cells, with no increase in the number of optic nerve astrocytes (W. Blakemore, personal communication). This was as expected because optic nerve astrocytes do not express PDGFRα.

It was possible that a slight increase in OLP numbers might not have been detected against the normal background of OLPs in wild-type nerves. We therefore investigated the effect of the *NSE–hPDGF-A* transgene in *PDGF-A* null mice [7], which have almost no OLPs in their optic nerves (Figure 4b), as a result of which they remain practically unmyelinated [5]. We crossed the *NSE–hPDGF-A* transgene into the *PDGF-A* null background but this did not result in any rescue of OLP numbers in the optic nerve whatsoever (Figure 4d). As a control, we analysed OLPs in the spinal cord, which, in contrast to the optic nerve, contains neuronal cell bodies as well as axon tracts. As previously reported, there were increased numbers of progenitor cells in spinal cords of *NSE–hPDGF-A* mice compared with wild-type mice ([6] and Figure 4e,g). In *PDGF-A* null mice, there were very few OLPs (< 5% of normal; [5,6] and Figure 4f). On top of

this background, the *NSE–hPDGF-A* transgene restored OLP numbers in the spinal cord (Figure 4h). Most of the OLPs were in the central grey matter of the cord where the neuronal cell soma are located. This confirms that transgene-derived PDGF-AA can be released from neurons and can stimulate OLP division.

Our results indicate that PDGF-AA is released from neuronal cell bodies but not from axons. This is consistent

There is immunohistochemical evidence that PDGF is present in growth cones of neurons in the developing