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

Marcus Fruttiger*, Andrew R. Calver† and William D. Richardson*

Neurons synthesise and secrete many growth and survival factors but it is not usually clear whether they are released locally at the cell body or further afield from axons or axon terminals. Without this information, we cannot predict the site(s) of action or the biological functions of many neuron-derived factors. For example, can neuronal platelet-derived growth factor (PDGF) be secreted from axons and reach glial cells in nerve-fibre (white-matter) tracts? To address this question, we expressed PDGF-A in retinal ganglion neurons in transgenic mice and tested for release of PDGF from cell bodies in the retina and from axons in the optic nerve. In both the retina and optic nerve, there are glial cells that express PDGF receptor α (PDGFRα) [1] and divide in response to PDGF [2–5], so we could detect functional PDGF indirectly through the mitogenic response of glia at both locations. Expressing PDGF-A in neurons under the control of the neuron-specific enolase promoter (*NSE–PDGF-A*) resulted in a striking hyperplasia of retinal astrocytes, demonstrating that PDGF is secreted from the cell bodies of neurons in the retina [4]. In contrast, glial proliferation in the optic nerve was unaffected, indicating that PDGF is not released from axons. When PDGF was expressed directly in the optic nerve under the control of an astrocyte-specific promoter (*GFAP–PDGF-A*), oligodendrocyte progenitors hyperproliferated, resulting in a hypertrophic optic nerve. We conclude that PDGF is constitutively secreted from neuronal cell bodies *in vivo*, but not from axons in white-matter tracts.

Addresses: *Wolfson Institute for Biomedical Research and Department of Biology, The Cruciform Building, University College London, Gower Street, London WC1E 6AU, UK. †New Frontiers Science Park North, SmithKline Beecham Pharmaceuticals, Third Avenue, Harlow, Essex CM19 5AW, UK.

Correspondence: Marcus Fruttiger
E-mail: m.fruttiger@ucl.ac.uk

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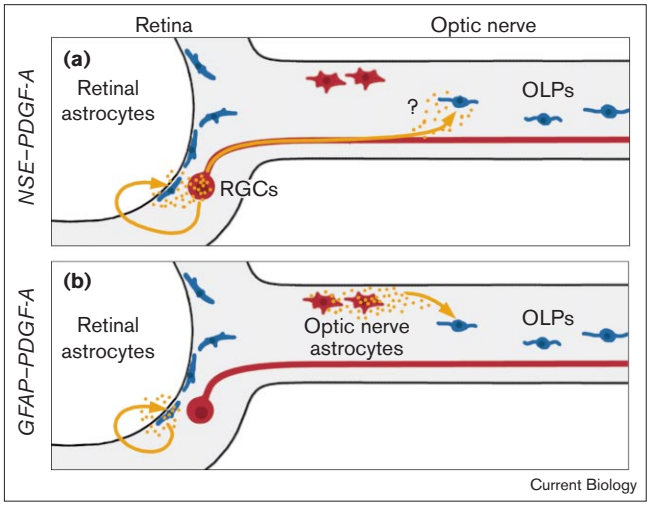
Results and discussion

Retinal ganglion cells (RGCs) — the neurons that project axons through the optic nerve — express PDGF-A [1] but it is not known whether they target PDGF to their axons or, if so, whether they can release PDGF into the nerve. Resident optic nerve astrocytes also synthesise PDGF-A [1]. It is difficult to establish whether RGCs or optic nerve astrocytes normally deliver PDGF-AA (the secreted dimer of PDGF-A) to the optic nerve because growth factors typically act at low concentrations and are difficult to trace once they are released into the extracellular milieu. We bypassed this difficulty by using a physiological measure of PDGF activity *in vivo*. We overexpressed PDGF-A either in neurons or astrocytes in transgenic mice and studied the proliferative response of PDGF-responsive glial cells. We monitored oligodendrocyte progenitors (OLPs) in the optic nerve and astrocytes in the retina; both these cell types express PDGFRα [1], allowing us to identify and count them by *in situ* hybridisation with a PDGFRα probe.

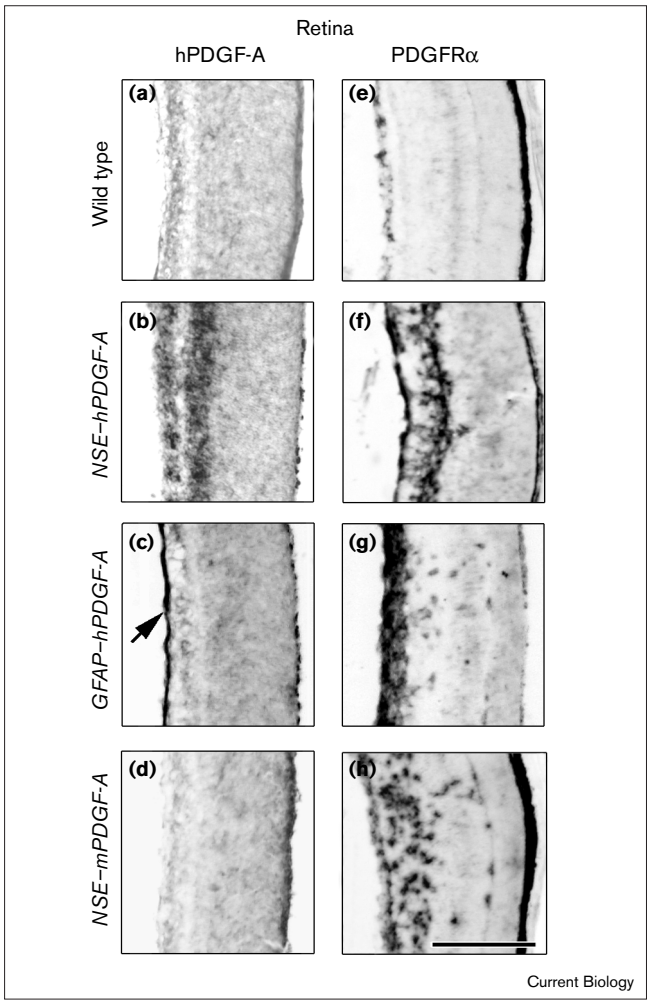
The pre-existing *NSE–hPDGF-A* transgenic mice [4,6] express human PDGF-A under the control of the mouse *NSE* promoter (Figure 1). In case human PDGF-AA might be targeted or secreted abnormally in mouse cells, we generated a new line that overexpresses mouse PDGF-A (*NSE–mPDGF-A*). We also generated control mice expressing human PDGF-A in astrocytes under the

Figure 1



Possible sites of PDGF-A release in transgenic mice. Cells carrying the PDGFRα are drawn in blue and PDGF-AA-secreting cells are in red. **(a)** Overexpression of PDGF-A (yellow) under the control of the *NSE* promoter resulted in neuron-specific expression, whereas **(b)** the *GFAP* promoter directed transgene expression to astrocytes. Note that retinal astrocytes express PDGFRα but not PDGF-A, whereas optic nerve astrocytes express PDGF-A but not PDGFRα [1].

Figure 2



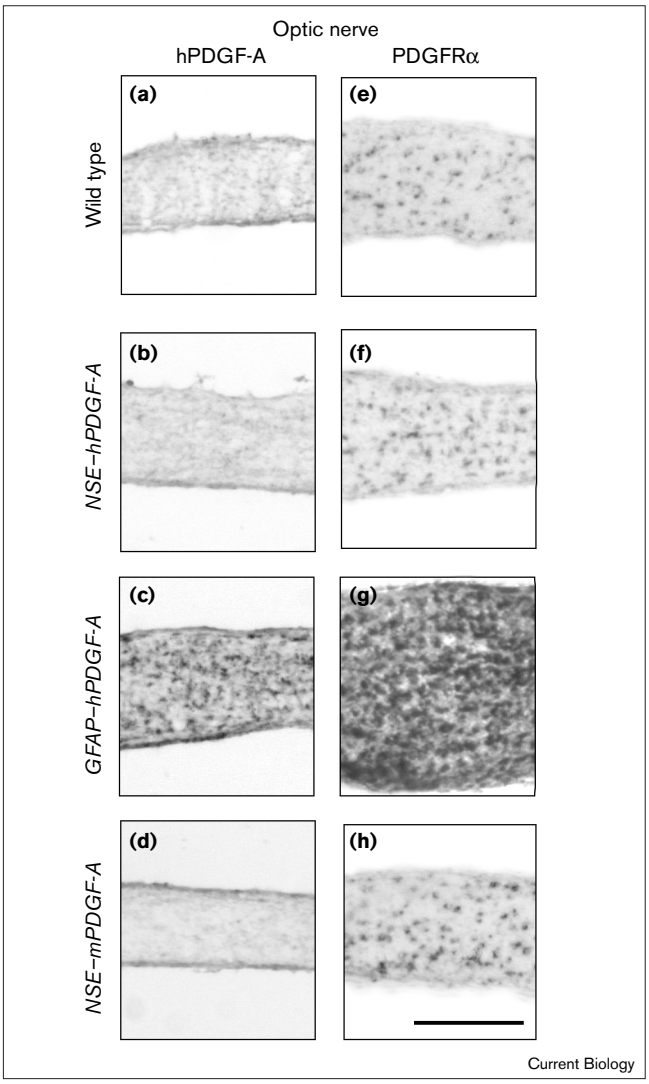
Hyperplasia of retinal astrocytes indicates the presence of transgenic *PDGF-A* in the retina. Cross sections of retinae from **(a–d)** 1 day old (P1) and **(e–h)** 5 day old (P5) mice that were (a,e) wild type, or expressed the (b,f) *NSE–hPDGF-A*, (c,g) *GFAP–hPDGF-A* or (d,h) *NSE–mPDGF-A* transgenes. The sections were probed for expression of (a–d) human *PDGF-A* or (e–h) *PDGFRα*. In (c), note the retinal astrocytes expressing human *PDGF-A* (arrow). In (d), note that the probe against human *PDGF-A* does not recognise endogenous mouse *PDGF-A*. Retinal astrocytes displayed hyperplasia in (f–h) transgenic mice, but not in (e) wild-type mice. The scale bar in (h) represents 200 μm.

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

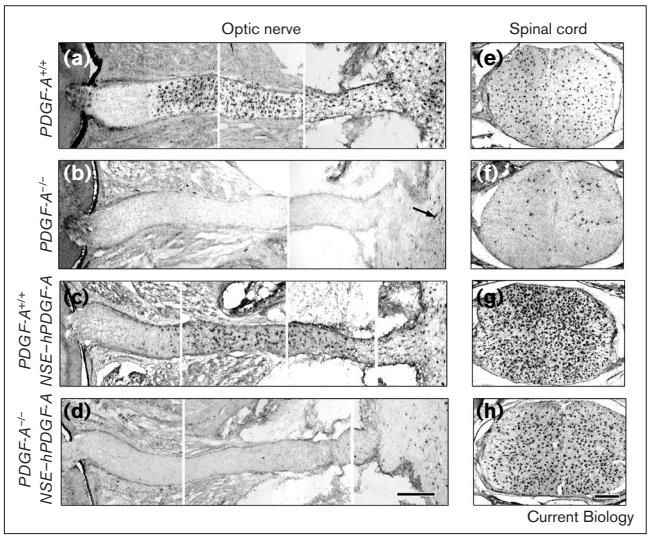
Figure 3



Transgenic PDGF-A increases OLP numbers in the optic nerve only in *GFAP–PDGF-A* but not in *NSE–PDGF-A* mice. Cross-sections through the optic nerves of **(a,e)** wild-type mice, or mice expressing the **(b,f)** *NSE–hPDGF-A*, **(c,g)** *GFAP–hPDGF-A* or **(d,h)** *NSE–mPDGF-A* transgenes. (a–d) *In situ* hybridisation with a probe against human *PDGF-A* confirmed the presence of transgene mRNA in the optic nerve of (c) *GFAP–hPDGF-A* mice but not in (b) *NSE–hPDGF-A* or in (d) *NSE–mPDGF-A* mice. (e–h) OLPs, visualised by *in situ* hybridisation with a mouse *PDGFRα* probe, were increased in number in (g) *GFAP–hPDGF-A* mice but not in (f) *NSE–hPDGF-A* or (h) *NSE–mPDGF-A* mice. There was no difference between the numbers of *PDGFRα*$^+$ cells per section in (e,f,h) but there was a large increase in (g). The scale bar in (h) represents 200 μm.

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.

Figure 4



In *PDGF-A* null mice, transgenic PDGF-A expressed in neurons can restore OLP numbers in the spinal cord (which contains both axons and neuronal cell bodies) but not in the optic nerve (which contains axons but no cell bodies). **(a–d)** *In situ* hybridisation with a probe against *PDGFRα* to visualise OLPs in longitudinal sections of P5 optic nerves. The entire length of the optic nerve was reconstructed from several adjacent sections. In (a) wild-type mice, the optic nerve was populated by *PDGFRα*$^+$ OLPs, whereas they were absent from the optic nerve of (b) *PDGF-A* null mice, in spite of some *PDGFRα*$^+$ cells being present in the optic chiasm (arrow). Transgene-derived hPDGF-A expressed in neurons did not reach OLPs in the optic nerve, as there was no increase in the number of optic nerve OLPs in *NSE–PDGF-A* transgenic mice either in the (c) presence or (d) absence of endogenous PDGF-A (that is, wild-type or *PDGF-A* null background). **(e–h)** In contrast, transgenic hPDGF-A from neurons was able to reach OLPs in the spinal cord, as the presence of the *NSE–PDGF-A* transgene increased OLP numbers in a wild-type background (compare panels e and g) and restored near-normal OLP numbers to the *PDGF-A* null background (compare panels f and h). These data are consistent with PDGF-A release from neuronal cell bodies in the spinal cord but not from axons in the optic nerve. The scale bars in (d,h) represent 200 μm.

Our results indicate that PDGF-AA is released from neuronal cell bodies but not from axons. This is consistent with the fact that cultured endothelial cells secrete PDGF-BB almost exclusively from the basal surface [8], as the basolateral domain of endothelial cells is thought to be equivalent to the somatodendritic region of neurons. It therefore appears that, in the optic nerve, OLP proliferation is entirely dependent on astrocyte-derived PDGF-AA. This conclusion is also supported by the fact that intraocular injection of colchicine, which blocks fast axonal transport, does not affect OLP proliferation in the optic nerve [9]. On the other hand, blocking electrical activity in RGC axons inhibits OLP proliferation in the optic nerve [10]. Taking that result together with the data described here it seems likely that, as suggested by Barres and Raff [11], an activity-dependent signal from RGC axons stimulates the secretion of mitogens, including PDGF-AA, from astrocytes in the optic nerve.

There is immunohistochemical evidence that PDGF is present in growth cones of neurons in the developing CNS [12]. This implies that PDGF is transported in an anterograde direction along axons. We were previously unable to detect PDGF-A immunoreactivity in RGC axons in the optic nerve although very weak immunoreactivity was present in RGC cell bodies and proximal axons in the retina [1]. Our failure to detect PDGF in optic nerve axons might have been a technical problem resulting from low concentrations of PDGF, or it might have been due to epitope masking by protein–protein interactions. In any case, our present data demonstrate that, even if PDGF is transported through the optic nerve, it is not released from axons during transit. Other diffusible polypeptides, for example brain-derived neurotrophic factor (BDNF), are believed to be transported in an anterograde direction along axons and to be secreted from nerve terminals [13,14]. It will be interesting and important to determine how transport occurs and whether it is generally true that such factors are prevented from being released into axon tracts. This would have major implications for neuron–glial interactions in white-matter tracts.

Materials and methods

Transgenic mice

Production of *NSE–hPDGF-A* mice has been described [4]. *NSE–mPDGF-A* and *GFAP–hPDGF-A* mice were produced according to standard techniques [15]. The *NSE–mPDGF-A* transgene was constructed by replacing *hPDGF* with *mPDGF* [16] in the *NSE–hPDGF-A* vector. The *GFAP–hPDGF-A* transgene contains the *GFAP* promoter [17] and a human *PDGF-A* cDNA engineered to encode the 'short' alternative-splice isoform [18]. The *hPDGF-A* cDNA was taken from the same plasmid that was used for production of the *NSE–hPDGF-A* mice [4]. Five *GFAP–PDGF-A* founder mice were produced, of which two died before reaching sexual maturity. The remaining three founders were used to establish lines. The observable phenotypes were indistinguishable in all three lines (data not shown) but the results described in this paper are derived from one line only. With the *NSE–mPDGF-A* transgene, four founder mice were created. One of them expressed the transgene and was used to establish a line.

In situ *hybridisation and quantification of* OLP *numbers*

Cryosections were processed for *in situ* hybridisation with digoxigenin-labelled probes (human PDGF-A and mouse PDGFRα) as described previously [5]. OLPs were counted in five cross-sections of optic nerves from three different mice of each genotype and expressed as mean and standard deviation for each genotype.

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