Expression of growth factor receptors in injured nervous tissue. II. Induction of specific platelet-derived growth factor binding in the injured PNS is associated with a breakdown in the blood–nerve barrier and endoneurial interstitial oedema

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Summary

We have studied the expression of the platelet-derived growth factor (PDGF) receptors in the injured chick PNS using [125I]-iodinated PDGF as a radioactive probe to map autoradiographically the in situ distribution of specific [125I]PDGF binding.

Crush or transection of the sciatic nerve led to a rapid and massive induction of specific [125I]PDGF binding on fibroblast-like cells of the injured endoneurium, already observed 2 h postoperatively. It is initially characterized by a symmetrical appearance both below and above the site of injury, spreading throughout the distal part of the lesioned nerve 1 to 2 days postoperatively. Comparison with distribution of specific [125I]β-nerve growth factor (β-NGF) binding (see preceding paper) revealed a number of important differences: unlike the specific [125I]β-NGF binding, which rapidly disappears after reinnervation of the distal nerve, this was not observed in the case of [125I]PDGF binding. [125I]PDGF binding also correlated poorly with the extent of axonal injury. The segmental removal of the perineurium, resulting in heavy interstitial oedema without widespread axonal injury, led to a strong, local induction of [125I]PDGF binding, while causing moderate β-NGF binding to only the few degenerating nerve fibre tubes. These results suggest the existence of different pathophysiological mechanisms that regulate the expression of PDGF and β-NGF receptors in the lesioned and regenerating PNS.

Introduction

The platelet-derived growth factor (PDGF) is a well-characterized basic heterodimeric protein of 31 kDa molecular weight (Antoniades et al., 1979; Heldin et al., 1979) which acts as a powerful mitogen on glial and mesenchymal cells in culture (Ross et al., 1974; Antoniades et al., 1975; Heldin et al., 1977). It is normally stored in the alpha-granules of the circulating platelets (Ross et al., 1974; Antoniades et al., 1975, 1979; Heldin et al., 1977, 1979; Witte et al., 1978), which provide the richest source of this growth factor in the normal adult organism. PDGF is rapidly secreted into the extracellular space in the process of platelet degranulation during blood clot formation (Witte et al., 1978). The normal localization of this growth factor and its release during blood coagulation have suggested that this mitogen could play an important role in early wound healing and tissue regeneration by stimulating the proliferation of PDGF-sensitive peritraumatic cells, following injury-induced bleeding, coagulation and the release of the platelet-stored proteins into the wound area.

To learn more about the physiological role of this growth factor following injury, during wound healing and in regeneration, we have analysed the spatial and temporal pattern of PDGF receptor expression in the normal and injured chick PNS. To delineate the pathophysiological mechanisms involved in regulating PDGF binding in the injured nervous tissue, we have employed different forms of experimental peripheral nerve injury to study in detail the effects of axotomy and denervation, injury-induced oedema and the interruption of blood supply. In addition, we
also compared it with that of β-nerve growth factor (β-NGF) binding (see Part I, Raivich & Kreutzberg, 1987).

Materials and methods

The materials, surgical procedures, preparation of tissue sections and the autoradiographic binding protocol used in this report were identical to those described in Part I. 125I-Iodinated human PDGF ([125I]PDGF; specific activity, 20–60 cpn pg⁻¹) and unlabelled PDGF used to label specific PDGF binding sites in situ were a generous gift of Dr Carl Henrik Heldin (Department of Medical and Physiological Chemistry, University of Uppsala). The tissue sections were incubated in 0.1 nm [125I]PDGF in phosphate-buffered saline, supplemented with 1 mg ml⁻¹ bovine serum albumin either in the absence or presence of 20 nm unlabelled PDGF (added to suppress the high-affinity [125I]PDGF binding). Sections incubated with added unlabelled PDGF served as controls for the non-specific and low-affinity binding. The level of the non-specific [125I]PDGF binding is shown in Fig. 1B and C. No competition for the specific [125I]PDGF binding was observed following the addition of 200 nm unlabelled mouse β-NGF or 20 μM insulin (Sigma, Munich).

Quantitative autoradiography of [125I]PDGF binding (see Fig. 2) was performed as described in the preceding paper, on X-ray films exposed for 18 h to the radioactive tissue sections. To determine the affinity of the specific [125I]PDGF binding, tissue sections of the sciatic nerve, 1 day after crush, were incubated with 0.5 ng ml⁻¹ [125I]PDGF and increasing amounts of unlabelled PDGF (0.4–100 nm). A sharp drop in the X-ray film optical density was observed between 1.5 and 6 ng ml⁻¹ unlabelled PDGF, with halff maximal competition at 4.1 ng ml⁻¹. From these data an apparent Kd of 0.12 nm was calculated. This affinity is in the middle of the range of Kd values reported for the PDGF receptor which seem to vary from 10 pM to 1 nM (Heldin et al., 1981; Bowen-Pope & Ross, 1982; Huang et al., 1982; Williams et al., 1982).

Surgical procedures

Sciatic nerve crush or resection was carried out as previously described in Part I. The resected nerve segment was displaced in an artificial muscle pouch in the quadriceps femoris muscle. Removal of the perineurial sheath was performed on a segment of the sciatic nerve, 1 cm long. When successful, this procedure did not result in a readily observable motor or sensory deficit.

Results

PDGF receptor expression following axotomy of the sciatic nerve

Intense [125I]PDGF binding in the nervous system of the embryonic and postnatal chick is normally restricted to the meninges, the pial septa of the CNS and the radicular parts of the peripheral nerves (see Fig. 1A). By contrast, only very low levels of specific [125I]PDGF binding are found in the endoneurium of embryonic (not shown) or undamaged, postnatal peripheral nerves (see for example Fig. 3A). This normal pattern in the distribution of specific [125I]PDGF binding is modified after injury to the PNS tissue. As early as 30 min following sciatic nerve crush, moderate [125I]PDGF binding is observed in the gap between the distal and proximal ends of the endoneurial tissue, which become separated (see Fig. 1B). The level of the non-specific PDGF binding to an adjacent section of the same nerve is shown in the lower part of Fig. 1B, where high-affinity binding was inhibited by coincubation of 20 nm unlabelled PDGF.

Increased specific [125I]PDGF binding to the distal and proximal endoneurium of the injured sciatic nerve is first detected at 2 h following crush. It rapidly increases, reaching a first, transient plateau at 24 h (Fig. 2). As shown in Fig. 1C, the specific [125I]PDGF binding is characterized by a symmetrical, bimodal pattern of distribution. Under high magnification, heavy autoradiographic labelling is observed on many small, longitudinally arranged, round to oval endoneurial cell profiles located between the longitudinal cords of unlabelled nerve fibre bundles (Figs 1D, 3D, 4D). Heavy labelling is also present on the subendothelial mesenchyme of the endoneurial vessels and on the subperineurial connective tissue (Fig. 1D). In the distal part of the sciatic nerve subjected to Wallerian degeneration, specific [125I]PDGF binding is not observed on the Büngner bands of denervated Schwann cells, but rather on the ensheathing endoneurial connective tissue (see Figs 3D, 4D).

Beginning at day 1 following crush, the region of heavy and specific endoneurial [125I]PDGF binding rapidly extends throughout the whole distal nerve. At day 2 it is already present on the distal cutaneous and muscular nerve branches of the denervated limb (Fig. 3B), becoming maximal approximately 3 weeks after crush (see Figs 2, 3C). Figs 1F and 2 show that high levels of [125I]PDGF binding to the distal part of the sciatic nerve are still maintained for a very long time period after the original injury.

Although massive [125I]PDGF binding is also rapidly induced on the adjacent, 1 cm wide, proximal sciatic nerve segment, this region of heavy autoradiographic labelling does not extend further from the site of injury. Instead, it gradually begins to recede. At 17 days postoperatively (DPO), specific [125I]PDGF binding to the proximal nerve is noted only on the 2 mm segment directly above the site of the crush (Fig. 1E).

The specific PDGF binding increases very much more slowly on the 1–2 mm wide distal and proximal nerve segments directly at the site of the axotomy (Fig. 1C, D). As shown in Fig. 2 it reaches and then slightly surpasses the levels observed 3 mm distally to the injury 4 days after crush. Interestingly, the PDGF