Regeneration and retrograde degeneration of axons in the rat optic nerve

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Summary

The retinal stump of the rat optic nerve was examined histologically 1-64 weeks after intracranial section of the nerve with or without grafting of autologous peripheral nerve segments. Single unmyelinated axons and bundles of unmyelinated axons appeared in cut optic nerves and were most abundant 2-4 weeks after section. With light and electron microscope radioautography after injection of tritiated amino acids into the globe, it was confirmed that many unmyelinated fibres arose from the optic nerve rather than from nearby peripheral nerves and it was estimated that some axons regenerated as far as 0.5 mm. At or near the end of retinofugal axons, structures resembling growth cones were seen at 2 weeks and vesicle-containing swellings similar to synapses were found at 1-2 months. Outgrowth from optic nerve axons was not obviously enhanced by peripheral nerve grafts although a few retinofugal axons became ensheathed by Schwann cells. Retrograde axonal degeneration was rapid in both cut and grafted optic nerves, the number of nerve fibres near the globe falling to less than 10% of normal after 4 weeks. A few myelinated and unmyelinated fibres were still present 64 weeks after nerve transection. In conclusion, some cut axons in the rat optic nerve display a transient regenerative response before undergoing retrograde degeneration.

Introduction

The rat optic nerve, an accessible well-characterized fibre tract (Hughes, 1977; Fukuda et al., 1982) with axons of almost uniform origin and direction of passage (Itaya, 1980) is a convenient site for studies of axonal degeneration and regeneration in the C.N.S. Axonal regeneration after section of the optic nerve in adult mammals is negligible compared to regrowth of axons in the optic nerve of fishes and amphibians (Reier & Webster, 1974; Murray, 1976; Lanners & Grafstein, 1980) or aberrant growth induced in developing mammalian retinofugal axons (Schneider, 1973; Lund & Harvey, 1981).

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However, abortive sprouting has been described in silver-stained specimens removed soon after injury to the optic nerve or retina of birds and mammals (Ramon y Cajal, 1928; Cankovic, 1968; Muchnick & Hibbard, 1980; Goldberg & Frank, 1980). From examination of the retina (James, 1933; Leinfelder, 1938; Mantz & Klein, 1951; Eayrs, 1952; Stone, 1966; Perry, 1981; Misantone et al., 1981; Grafstein & Ingoglia, 1982) or the optic nerve (Ramon y Cajal, 1928; Leinfelder, 1938; Lin & Ingram, 1973; Quigley et al., 1977), it is known that extensive retrograde degeneration follows optic nerve transection in mammals. In the present study, responses to transection of axons in the retinal stump of the rat optic nerve have been examined with ultrastructural, radioautographic and quantitative techniques.

Results of recent experiments support the hypothesis (Ramon y Cajal, 1928) that the regrowth of at least some axons depends upon the neuroglial environment at the axonal tip. In vivo, many peripheral (Aguayo et al., 1979; Stensaas et al., 1979; Weinberg & Spencer, 1979), spinal (Richardson et al., 1980, 1982a, 1982b) and cerebral (David & Aguayo, 1981; Benfey & Aguayo, 1982) axons regenerate further within peripheral nervous tissue than within central nervous tissue. In vitro, nerve growth activities are demonstrable in peripheral nervous tissue (Varon et al., 1981; Richardson & Ebendal, 1982). A second aim of this study was to examine the influence of peripheral nerve grafts on the regeneration of retinofugal axons.

Methods

Long-Evans rats weighing approximately 150 g were used for all experiments. Rats were anaesthetized with pentobarbital and operations performed under aseptic conditions with the use of an operating microscope and bipolar cautery. After a frontal craniectomy and partial frontal lobectomy, the optic nerve was transected intradurally between the optic foramen and optic chiasm in 34 rats. Operations were performed intracranially in order to avoid injury to the central retinal artery which enters the nerve in the orbit. In an additional 39 animals, segments from the tibial or common peroneal nerve of the same rat were transplanted to the operative site. In some rats, a short peripheral nerve graft (3 mm long) was interposed between cut ends of the optic nerve; in other animals, a longer segment (5-10 mm long) was laid on the dura over the anterior cranial floor with the medial end in contact with the retinal stump of the optic nerve. To minimize trauma to the optic nerve, stitches were not used to maintain apposition.

For routine histological examination, 35 animals were perfused with fixative (1.5–2.5% glutaraldehyde, 2.0–0.5% paraformaldehyde in 0.1 M Sörensen’s phosphate buffer, pH 7.4). The retinal stump of the optic nerve, approximately 8 mm long, was removed and usually divided into four segments. Specimens were fixed several hours, rinsed, post-fixed in osmium tetroxide, dehydrated and embedded in Epon. One-micron sections for light microscopy were stained with toluidine blue; thin sections for electron microscopy were stained with lead citrate and uranyl acetate. To determine the total number of myelinated axons in optic nerves, the density of fibres in deep and superficial regions was established by counting fibres in grid squares under oil immersion light microscopy (× 2000) and the area of each portion was measured with a drawing tube and planimeter. Unmyelinated axons were counted from electron micrographs or directly under the electron microscope. The number of unmyelinated axons in a cross-section was usually calculated by estimating the ratio of unmyelinated to myelinated axons in several zones and counting the total number of myelinated axons. In cross-sections where unmyelinated axons were