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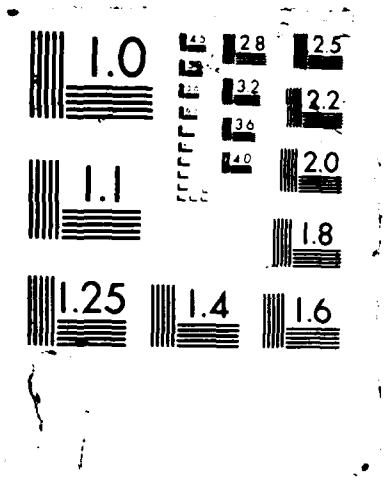
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Three Proteins Made By Glial Cells of the Goldfish (C. auratus) Optic Nerve Undergo Increased Synthesis Following Trauma to the Optic Nerve

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Glial cells have been shown to increase the levels of synthesis of selected proteins in response to damage inflicted upon their associated axons. These proteins may be instrumental in mediating axonal repair. In this study proteins synthesized by the glial cells of the goldfish optic nerve were examined using tissue incubation, polyacrylamide gel electrophoresis, and gel fluorography. Ten days after monocular nerve crush or monocular enucleation, three glial proteins were found to have increased levels of synthesis relative to controls. These enhanced glial proteins, or FGPs, were detectably increased relative to controls at 4.5 hr after nerve trauma, and returned to control levels of synthesis by 6 months post-crush. The FGPs displayed apparent molecular weights of 16, 30, and 42 kilodaltons (i.e. FGP-16, FGP-30, and FGP-42).

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ABSTRACT

Glial cells have been shown to increase the levels of synthesis of selected proteins in response to damage inflicted upon their associated axons. These proteins may be instrumental in mediating axonal repair. In this study proteins synthesized by the glial cells of the goldfish optic nerve were examined using tissue incubation, polyacrylamide gel electrophoresis, and gel fluorography. Ten days after monocular nerve crush or monocular enucleation, three glial proteins were found to have increased levels of synthesis relative to controls. These enhanced glial proteins, or EGPs, were detectably increased relative to controls at 4.5 hr after nerve trauma, and returned to control levels of synthesis by 6 months post-crush. The EGPs displayed apparent molecular weights of 16, 30, and 42 kilodaltons (i.e. EGP-16, EGP-30, and EGP-42).

INTRODUCTION

Proteins manufactured by glial cells may subserve important functions during the processes of both neuronal maturation and nerve regeneration. Studies in this area have demonstrated that various glial cell preparations can exert a positive effect on nerve growth [1-6]. More recently, specific glial proteins have been identified which are thought to be instrumental to the mechanisms of axon growth and regeneration [7, 8]. We have studied the effects of nerve crush and enucleation on the synthesis of proteins manufactured by glial cells of the goldfish (C. auratus) optic nerve. The results indicate that the response of goldfish optic nerve glial cells to nerve trauma includes the increase in synthesis of three soluble proteins.

METHODS

Goldfish received either unilateral intraorbital optic nerve crush or monocular enucleation. Ten days or 6 months later the crushed optic nerve was excised, minced, and incubated at room temperature in a protein-free isotonic medium [9] (100 ul per nerve) which also contained 60 uCi of 35S-methionine (35S-met). At the end of 2 hr, the mixture was centrifuged at 13,000 x g for 5 min to sediment the tissue fragments. The medium (supernatant) was then drawn off and brought to a concentration of 0.01 mg/ml with bovine serum albumin (BSA). Proteins in the medium were precipitated by adding an equal volume of ice-cold 10% trichloroacetic acid (TCA) and centrifuging at 13,000 x g for 15 min. The resulting pellet was washed with diethyl ether to rid the sample of excess TCA and solubilized in 50 ul of electrophoresis sample buffer in preparation for one-dimensional (SDS-PAGE) [10] or two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) [11] analysis. Radiolabelled proteins that had been synthesized by the optic nerve's glial cells during incubation were made visible using gel fluorography [12, 13]. Optic nerves from enucleates were excised 10 days post-enucleation and were manipulated as above. Controls for these procedures included the identical manipulation of undamaged contralateral optic nerves and optic nerves from normal fish. Three animals were used for each experimental and control condition.

It was also considered necessary to demonstrate that the incubation conditions were capable of supporting protein synthesis in glial cells throughout the 2-hr

incubation period. To that end a synthesis viability test was performed. This test consisted of subjecting normal optic nerves to a 2.5-hr pre-incubation in unlabelled medium prior to the addition of 35S-met. After introducing the radiolabel, the nerves were incubated an additional 2 hr, and then manipulated as described above.

RESULTS

The paradigm was designed to examine the response of optic nerve glia to nerve crush or enucleation in terms of protein synthesis. Since the optic axons were separated from their cell bodies at the time of excision, the optic nerve's glial cells were the only significant entities capable of incorporating 35S-met into protein during the incubation step. At 10 days post-crush three proteins were found whose levels of synthesis were visibly elevated relative to controls, as shown in Figure 1B. For convenience, these are referred to as enhanced glial proteins, or EGPs, and each is named according to its apparent molecular weight in kilodaltons (i.e. EGP-16, EGP-30, and EGP-42). The synthesis of each EGP at 6 months post-crush was undiscernible from control levels; the electrophoretic profiles were indistinguishable from those of normal nerves shown in Figures 1A and 2A.

Enucleation also increased the synthesis of all three EGPs, as shown in Figure 2B. This suggests that the trigger mechanism(s) that initiates their increased synthesis responds to nerve trauma rather than some signal specifically associated with nerve regeneration. This result does not mean, however, that EGPs do not function in some process crucial to axon repair. The effects of long-term enucleation (6 months or longer) on EGP synthesis were not examined since too little of the optic nerve remained at that point to acquire a suitable tissue sample.

The synthesis viability test demonstrated that the incubation conditions supported protein synthesis since the glial cells of the excised nerves were capable of incorporating 35S-met into protein even after a 2.5-hr pre-incubation in unlabelled medium, as demonstrated in Figure 2C. While initially designed simply as a control measure, this test also gave the interesting result that normal optic nerves began to synthesize the EGPs in small, yet detectably elevated amounts sometime within 4.5 hr of their excision. This is a reasonable result since excision of the nerve subjects it to significant trauma. Furthermore, it indicates that the biochemical switch(es)

that initiates the elevation of synthesis of the EGPs acts very rapidly.

DISCUSSION

This study demonstrated that the glial cells associated with goldfish optic nerve axons respond to nerve crush and enucleation, in part, by increasing the levels of synthesis of three proteins, EGP-16, EGP-30, and EGP-42. The synthesis of these proteins returned to normal control levels in the nerve crush paradigm by 6 months post-crush. This time course of events suggests that the EGPs may function in some phase of axon repair [14]. What these roles might be has been extensively discussed elsewhere [1-8, 15, 16], and should become clearer as the identities of the EGPs, and their homologues in other species, are made known. For instance, it is likely, based on studies performed in the rat and rabbit, that EGP-30 is (or is similar to) apolipoprotein-E (J.A. Freeman, personal communication). That glial factors can potentiate nerve growth and regeneration is well documented, and it may be that the EGPs are responsible for at least a portion of these effects.

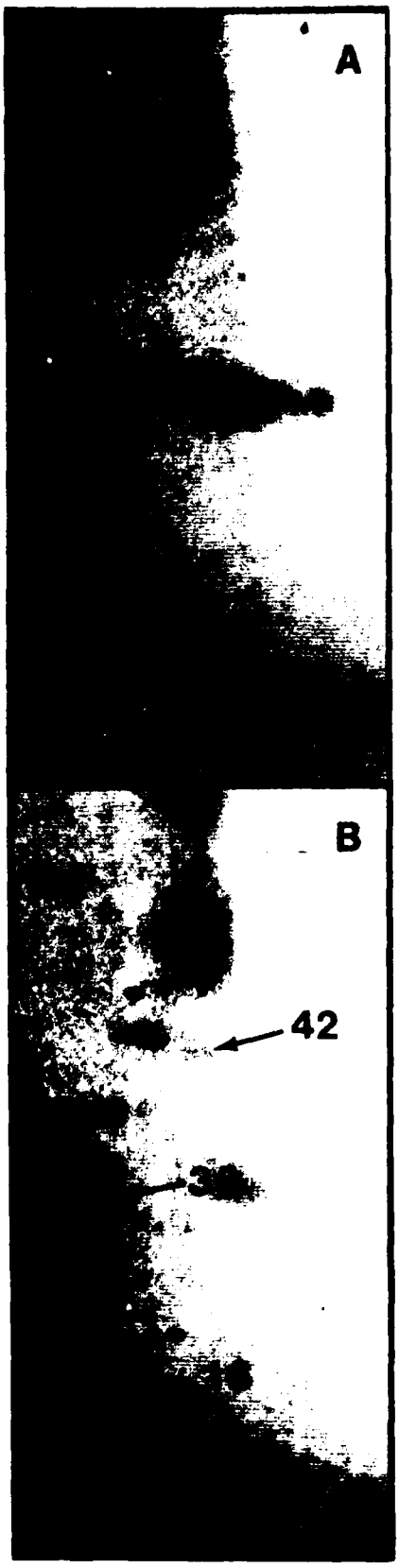

Figure 1. The goldfish optic nerve glia respond to nerve crush by increasing the levels of synthesis of EGPs. This figure shows 2-D PAGE/fluorography preparations of radiolabelled proteins synthesized by optic nerve glia and subsequently emitted into the medium during incubation. A, normal control. B, 10 days post-crush.

Three enhanced glial proteins, or EGPs, appear in the gels from crushed nerve preparations, suggesting that these polypeptides undergo enhanced synthesis as a result of the trauma sustained by the optic nerve. Similar results are obtained 10 days after enucleation.

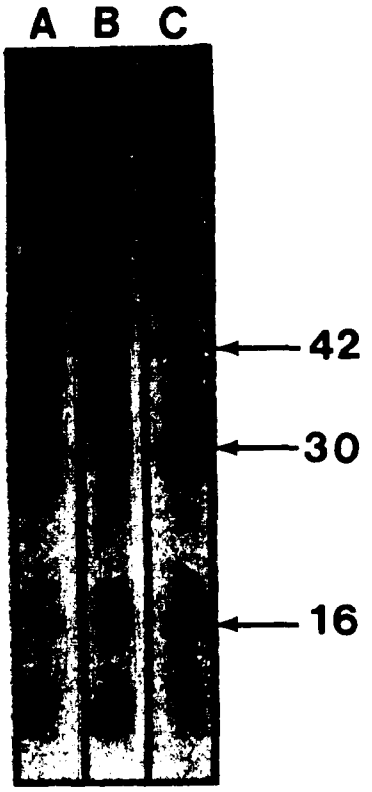

Figure 2. Various conditions result in the elevated synthesis of EGP-16, EGP-30, and EGP-42. This figure shows SDS-PAGE/fluorography preparations from three manipulations of the optic nerve. A, normal control. B, 10 days post-enucleation. C, normal nerve which received a 2.5-hr pre-incubation in unlabelled medium prior to the addition of 35S-met.

EGP-16 and EGP-42 appear as faint bands in SDS-PAGE preparations. As can be seen in Figure 1, EGP-30 has a more basic 29 kilodalton (KD) neighbor which makes EGP-30 difficult to identify using SDS-PAGE. Characteristic broadening of the 28-31 KD band, as seen in lanes B and C, signals the induction of EGP-30 synthesis in these preparations. The synthesis of all three EGPs is increased as a result of both nerve crush and enucleation. Simply pre-incubating a normal optic nerve for 2.5 hr is also sufficient to cause perceptible increases in the levels of the EGPs to occur.

NOTES ON FIGURES

The polyacrylamide gels shown in Figures 1 and 2 are not really gels, but photographs of computer-driven reconstructions of gels. Radiolabelled proteins of glial origin were made visible using gel fluorography (13). The fluorograms were digitized and stored in the memory of a PDP 11-70 computer. The digitized image was then subjected to a 256 x 256 fast Fourier transformation (FFT), and the results were added back to the original image to enhance subtle shades of gray. The image then underwent a 9-point smoothing routine to restore normal border effects around the enhanced images. The reconstructed fluorograms were then displayed on a high resolution video monitor and photographed on high contrast film to produce the figures.

The algorithms for this procedure were designed by Mr. Larry Sherman and Dr. Harry Zwick, Letterman Army Institute of Research.

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