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Retinal Derived Growth Factor: A Regulator of Neural Regeneration and Revascularization in Wound Healing

Annual/Final Report

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Retinal Derived Growth Factor (a heparin binding growth factor that is identical to aFGF (acidic Fibroblast Growth Factor)) stimulates neurite outgrowth in vitro. We have established a model system that will allow us to determine if a gradient of FGF can cause nerve regeneration in vivo and that this regeneration is predominantly due to increased neurite outgrowth by sensory nerves.

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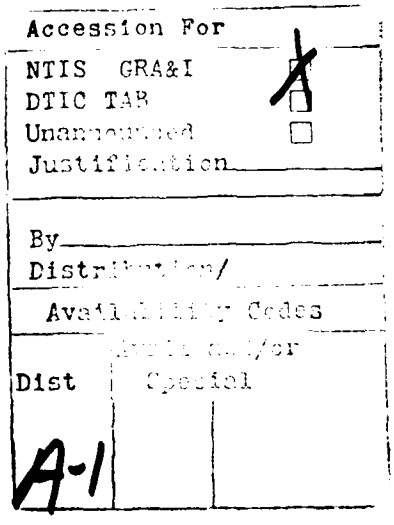
Summary

Acidic Fibroblast Growth Factor (aFGF) is a peptide which has been purified from a number of tissues, but which is present at very high concentration in the central nervous system. This growth factor is now emerging as an important neurotrophic agent that has effects on both cell survival and neurite extension by a number of nerve types. Experiments supported by this contract were designed: 1) to determine whether aFGF could act as a neurotropic factor (i.e., a factor that would be able to influence the directionality of neurite outgrowth by influencing nerves to grow toward a source of aFGF), or 2) if it could stimulate nerve regeneration in vivo. The experiments that have been done thus far do not provide any evidence that aFGF is a tropic factor; however, it is clear that aFGF can stimulate nerve regeneration in an in vivo model system. The potential therapeutic benefits of a regimen including aFGF remain to be evaluated.



Foreword

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

In conducting the research described in this report, the investigator adhered to the "Guide for the Care and Use of Laboratory Animals", prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

Table of Contents

Objective 1: To examine the effects of aFGF on the directionality of neurite extension by PC12 cells and sympathetic ganglia.

Rational and Experimental Design: We will determine aFGF (acidic Fibroblast Growth Factor) can influence the direction of neurite outgrowth from when it is slowly and continuously released from a polymer of ELVAX (ethylene vinal acetate).

Results: As reported in the first annual report, we have successfully prepared RDGF and incorporated it into a slow release ELVAX polymer. This system provided for a slow release of aFGF over a period of at least three days in a form that retained biological activity. Culturing of PC12 cells in the presence of such a polymer resulted in a stimulation of neurite outgrowth as would be expected; but, there was no directionality to this neurite outgrowth as measured in a number of different experiments. Thus we concluded that either aFGF does not function as a tropic or that the system that was established is not capable of maintaining an adequate gradient for this biological activity to manifest itself. Although this work did not provide any positive evidence for aFGF participating in a directional guidance mechanism, it would be a serious overinterpretation to suggest that we have excluded the possibility that aFGF might do so in some context. Because of the "negative" nature of the observations we made, it will not be possible to submit a manuscript describing these findings. We have, however, continued to explore other methods of establishing an FGF gradient and releasing FGF from substrates to determine these effects on neurite outgrowth.

Objective 2: To determine if aFGF will enhance the efficiency of nerve regeneration in vivo.

Work supported by this contract has demonstrated that aFGF enhances peripheral nerve regeneration in vivo. This work has been formulated into a manuscript and published. The paper was awarded the Peter J. Gingrass Memorial Award at the thirty-second annual meeting of the Plastic Surgery Research Conference.

Results: The sciatic nerve of Sprague-Dawley rats was transected and an entubulation repair was performed. Entubulation was performed either in the presence of collagen or collagen plus aFGF. The presence of aFGF clearly increased the number of myelinated axons that regenerated across a 5 mm nerve gap (figure 1). This increase in myelinated axons could result either from an increase in the number of nerves that were successfully capable of regenerating across the nerve gap or from a

bifurcation of axons. To distinguish these two possibilities and to determine the nature of the nerves that were regenerating across the nerve guide tube, we performed horseradish peroxidase labeling from a transection distal to the original repair. These studies confirm that there was an increase in the total number of HRP-labeled primary nerves in response to aFGF (figure 2). Interestingly, this increase was primarily due to an effect of aFGF on the ability of primary sensory neurons to regenerate through the nerve guide with a small and statistically insignificant effect on the motor nerves (figure 2). Thus we conclude that aFGF has the ability to stimulate the regeneration of sensory, but apparently not motor neurons.

Publications:

Cordeiro, P.G., Seckel, B.R., Lipton, S.A., D'Amore, P.A., Wagner, J.A.,, and Madison, R.D. (1989) Acidic fibroblast growth factor enhances peripheral nerve regeneration in vivo. J. of Plastic and Reconstructive Surgery, 83: 1013-1019.

Personnel supported by this contract:

John A. Wagner, Roger Madison, Patricia D'Amore, Brian Minsk, Holly Swanson, and Tom Horez.

No persons supported by this grant received a graduate degree during the period of support.

FIGURES:

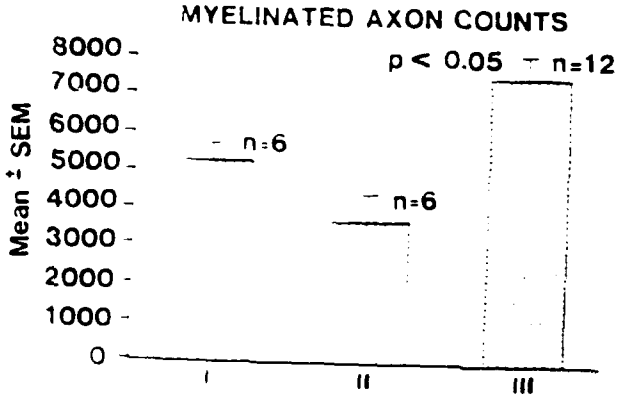


Fig. 1. A comparison of myelinated axon counts in nerve guides filled with collagen alone (group I, n=6) versus collagen plus heparin (group II, n=6) versus collagen plus heparin plus aFGF (group III, n=12).

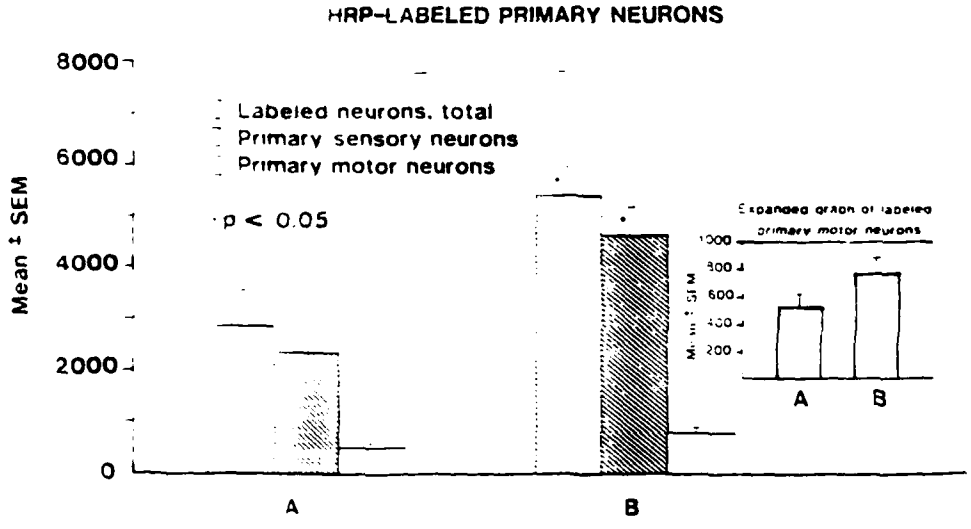


Fig. 2. A comparison of total horseradish peroxidase-labeled primary neurons, primary labeled sensory neurons, and primary labeled motor neurons in nerve guides filled with collagen plus heparin (group A, n=6) versus collagen plus heparin plus aFGF (group B, n=6).