GREAT LAKES FISHERY COMMISSION

Project Completion Report[1]

Lamprey Cell Lines for Studies of Cellular Endocrinology and Development of In Vitro Assays for Biological Lampricides

by

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Research Completion Report

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Abstract

We have developed methods for the culture of cells derived from tissues of adult and larval sea lamprey. Cultures were initiated from adult and larval liver, gill, gut and muscle and adult brain, heart, kidney and ovary. The lamprey cells were viable for more than four months in culture and cells from ovary, muscle, gut, gill and liver were propagated for multiple passages. Conditions for optimal cell attachment and spreading were established for each tissue. Cultures from all tissues except liver were initiated on surfaces pretreated with fibronectin and collagen. Optimal liver cell attachment was achieved on basement membrane. A large percentage of cells synthesizing DNA were detected in cultures maintained for more than 5 weeks indicating that the cells continued to proliferate in vivo. Ultrastructural examination by transmission electron microscopy revealed the accumulation of lipid in gill and liver cells and the presence of cilia in some of the cultures derived from brain. Plasmid DNA was introduced into the liver cells by transfection and transient expression of the foreign gene was detected. Cells derived from liver and ovary remained metabolically active for more than five weeks in culture, releasing many labeled proteins into the medium. The liver cells were maintained for several weeks in serum-free medium.

Cultures maintained as long as four months provide a system amenable to in vitro manipulations such as metabolic labeling, toxicity testing for the identification of potential lampricides and gene transfer studies. The cultures also provide an assay system for the identification and characterization of compounds influencing lamprey cell growth. Cultures derived from liver and gonad may provide an in vitro source of biologically active compounds such as hormones, growth factors and pheromones.

Introduction

At the start of this research project, lamprey cell cultures had not been derived from any tissues. Therefore, during the first year we worked to develop basic methods for the preparation of lamprey tissues for cell culture and to formulate a basal nutrient medium that will support the long-term growth of lamprey cells in vitro. During the second year of this project these methods were utilized to derive and characterize long-term cultures initiated from several tissues of adult and larval lamprey including liver, gill, ovary, kidney, heart, brain and muscle. The cells were examined for their ability to proliferate under various culture conditions including serum-free medium and the cells were examined ultrastructurally by electron microscopy. Due to the importance of liver and gonadal cell cultures for studies of factors influencing lamprey growth and reproduction, we examined the feasibility of using cultures from these tissues for the in vitro production of biologically active compounds and for gene transfer experiments.

Results

Culture conditions Cell cultures were derived from adult and larval liver, gill, muscle and gut and adult ovary, brain, heart and kidney. Primary cultures were viable for more than four months and cultures initiated from adult muscle, gill, liver, gut and ovary were propagated for two to three passages. Cultures were derived in LDF basal nutrient medium (Leibovitz's L-15, Dulbecco's modified Eagles and Ham's F12 media, 50:35:15). For all of the tissues except ovary, optimal cell growth was achieved in LDF supplemented with 10% FBS and 2% trout serum. Ovary cell cultures were maintained in lower serum concentrations (1% FBS and 1% trout serum) along with lamprey ovary extract (50 μg protein/ml) and insulin (10 μg/ml). Several other medium supplements were tested on the cultures including epidermal growth factor (EGF), trout embryo extract, bovine embryo fluid, lamprey serum and CPSR-3 (Sigma) serum replacement. Since bovine embryo fluid and CPSR-3 enhanced the growth and survival of cells derived from every tissue except ovary, these supplements were routinely added to the culture medium.

Various attachment factors including polylysine, fibronectin, collagen type IV and matrigel basement membrane (Collaborative Research, Minneapolis) were tested on the primary cultures. For all tissues except liver, optimal cell attachment and spreading was obtained by pretreating each flask with a combination of fibronectin and collagen. Optimal liver cell attachment and outgrowth was achieved on matrigel basement membrane. Polylysine improved cell attachment for each tissue but prevented spreading and growth. Plating efficiency was low for all cultures initiated in the absence of attachment factors.

Cell Morphology Primary cultures derived from each tissue were comprised of both fibroblastic and epithelial cell types. In most cultures both cell types continued to proliferate, however after several weeks only epithelial cells were present in primary cultures derived from liver and gut and only fibroblasts were present in muscle cell cultures. Explant cultures of brain tissue were maintained for a minimum of four weeks on collagen-fibronectin treated plates. Neurite outgrowth was observed in 24 hr-old cultures and clusters of ciliated cells were present in some of the cultures from brain. Beating cilia were detected in the cultures for more than three weeks and bundles of

microfilaments exhibiting the 9 X 2 configuration characteristic of cilia were identified by electron microscopy.

Characterization of the cultures To estimate the number of proliferating cells present in the long-term cultures, cells synthesizing DNA were identified by precursor incorporation. Cultures were labeled with the nucleotide analog, bromodeoxyuridine (BrdU) and cells incorporating BrdU were identified with a fluorescent labeled monoclonal antibody. The results demonstrated that a large number of proliferating cells were present in five week old cultures derived from adult and larval tissues. Labeled cells were present in areas of the cultures containing flat monolayers of cells and in cell aggregates.

Since the liver and gonad are a source of many biologically active compounds that control lamprey growth and reproduction, we were interested in determining if cell cultures derived from these tissues remain metabolically active in vitro and therefore may provide an in vitro source of these compounds. Five week-old cultures from ovary, liver and gill were metabolically labeled with [^{35}S] methionine and the labeled proteins synthesized and released by the cultured cells were examined by autoradiography. Conditioned medium was collected from the cultures and proteins contained in the medium were concentrated by ammonium sulfate precipitation. The precipitated proteins were separated by SDS polyacrylamide gel electrophoresis and visualized by autoradiography. These results revealed that the ovary and liver cell cultures continued to synthesize a large number of proteins that were released into the medium. The proteins ranged in molecular weight from over 200-kilodaltons (kD) to less than 20-kD.

To determine the feasibility of using the lamprey cell cultures for gene transfer experiments, we introduced foreign DNA into the cultured liver cells and screened the cells for expression of the foreign gene. Cells were transfected with a plasmid containing the β-galactosidase reporter gene under the control of the simian virus 40 (SV40) derived promoter. Cells expressing β-galactosidase turn blue in the presence of the substrate x-gal. Liver cells expressing the reporter gene were identified 4 days after transfection demonstrating that the viral derived regulatory sequences were functional in the lamprey cells.

Conclusion

We have developed methods to culture cells from tissues of adult and larval sea lamprey. The cultures were maintained up to four months and several of the cultures were propagated for multiple passages. The manipulation of lamprey cells in vitro is important for research leading to the identification and characterization of hormonal factors influencing lamprey growth and reproduction. We have demonstrated that liver and gonadal cells remain metabolically active for several weeks in culture providing a potential source of biologically important compounds such as growth factors and pheromones that regulate lamprey reproduction and growth. The cultures will also provide an in vitro assay system for the identification of potential lampricides and an experimental system for gene transfer experiments.