Neurobiology: Molecular Biological Approaches to Understanding Neuronal Function and Development

Editor

Paul O'Lague

NEUROBIOLOGY: Molecular Biological Approaches to Understanding Neuronal Function and Development

Proceedings of a UCLA Symposium
Held in Keystone, Colorado
April 1–7, 1984

Editor

PAUL O'LAGUE

Biology Department
University of California
Los Angeles, California

Alan R. Liss, Inc. • New York

Address all Inquiries to the Publisher
Alan R. Liss, Inc., 41 East 11th Street, New York, NY 10003

Printed in the United States of America.

Library of Congress Cataloging in Publication Data

Main entry under title:

Neurobiology—molecular biological approaches to understanding neuronal function and development.

Includes bibliographies and index.
1. Neurobiology—Congresses. 2. Developmental neurology—Congresses. 3. Molecular biology—Congresses.
I. O'Lague, Paul. II. University of California, Los Angeles.
[DNLM: 1. Cytoplasmic Filaments—congresses. 2. Ion Channels—congresses. 3. Molecular Biology—congresses. 4. Neurons—growth and development—congresses.
5. Neurons—physiology—congresses. W3 U17N v. 24/
WL 102.5 N4925 1984]
QP351.N4276 1985 612.8 85-4539
ISBN 0-8451-2623-7

UCLA Symposia on Molecular and Cellular Biology, New Series

Series Editor
C. Fred Fox

Volume 1
Differentiation and Function of Hematopoietic Cell Surfaces, Vincent T. Marchesi and Robert C. Gallo, *Editors*

Volume 2
Mechanisms of Chemical Carcinogenesis, Curtis C. Harris and Peter A. Cerutti, *Editors*

Volume 3
Cellular Recognition, William A. Frazier, Luis Glaser, and David I. Gottlieb, *Editors*

Volume 4
Rational Basis for Chemotherapy, Bruce A. Chabner, *Editor*

Volume 5
Tumor Viruses and Differentiation, Edward M. Scolnick and Arnold J. Levine, *Editors*

Volume 6
Evolution of Hormone-Receptor Systems, Ralph A. Bradshaw and Gordon N. Gill, *Editors*

Volume 7
Recent Advances in Bone Marrow Transplantation, Robert Peter Gale, *Editor*

Volume 8
Gene Expression, Dean H. Hamer and Martin J. Rosenberg, *Editors*

Volume 9
Normal and Neoplastic Hematopoiesis, David W. Golde and Paul A. Marks, *Editors*

Volume 10
Mechanisms of DNA Replication and Recombination, Nicholas R. Cozzarelli, *Editor*

Volume 11
Cellular Responses to DNA Damage, Errol C. Friedberg and Bryn A. Bridges, *Editors*

Volume 12
Plant Molecular Biology, Robert B. Goldberg, *Editor*

Volume 13
Molecular Biology of Host-Parasite Interactions, Nina Agabian and Harvey Eisen, *Editors*

Volume 14
Biosynthesis of the Photosynthetic Apparatus: Molecular Biology, Development and Regulation, J. Philip Thornber, L. Andrew Staehelin, and Richard B. Hallick, *Editors*

Volume 15
Protein Transport and Secretion, Dale L. Oxender, *Editor*

Volume 16
Acquired Immune Deficiency Syndrome, Michael S. Gottlieb and Jerome E. Groopman, *Editors*

Volume 17
Genes and Cancer, J. Michael Bishop, Janet D. Rowley, and Mel Greaves, *Editors*

Volume 18
Regulation of the Immune System, Harvey Cantor, Leonard Chess, and Eli Sercarz, *Editors*

Volume 19
Molecular Biology of Development, Eric H. Davidson and Richard A. Firtel, *Editors*

Volume 20
Genome Rearrangement, Ira Herskowitz and Melvin Simon, *Editors*

Volume 21
Herpesvirus, Fred Rapp, *Editor*

Volume 22
Cellular and Molecular Biology of Plant Stress, Joe L. Key and Tsune Kosuge, *Editors*

Volume 23
Membrane Receptors and Cellular Regulation, Michael P. Czech and C. Ronald Kahn, *Editors*

Volume 24
Neurobiology: Molecular Biological Approaches to Understanding Neuronal Function and Development, Paul O'Lague, *Editor*

Volume 25
Extracellular Matrix: Structure and Function, A. Hari Reddi, *Editor*

UCLA Symposia Published Previously

(Numbers refer to the publishers listed below.)

1972
Membrane Research (2)

1973
Membranes (1)
Virus Research (2)

1974
Molecular Mechanisms for the Repair of DNA (4)
Membranes (1)
Assembly Mechanisms (1)
The Immune System: Genes, Receptors, Signals (2)
Mechanisms of Virus Disease (3)

1975
Energy Transducing Mechanisms (1)
Cell Surface Receptors (1)
Developmental Biology (3)
DNA Synthesis and Its Regulation (3)

1976
Cellular Neurobiology (1)
Cell Shape and Surface Architecture (1)
Animal Virology (2)
Molecular Mechanisms in the Control of Gene Expression (2)

1977
Cell Surface Carbohydrates and Biological Recognition (1)
Molecular Approaches to Eucaryotic Genetic Systems (2)
Molecular Human Cytogenetics (2)
Molecular Aspects of Membrane Transport (1)
Immune System: Genetics and Regulation (2)

1978
DNA Repair Mechanisms (2)
Transmembrane Signaling (1)
Hematopoietic Cell Differentiation (2)
Normal and Abnormal Red Cell Membranes (1)
Persistent Viruses (2)
Cell Reproduction: Daniel Mazia Dedicatory Volume (2)

1979
Covalent and Non-Covalent Modulation of Protein Function (2)
Eucaryotic Gene Regulation (2)
Biological Recognition and Assembly (1)
Extrachromosomal DNA (2)
Tumor Cell Surfaces and Malignancy (1)
T and B Lymphocytes: Recognition and Function (2)

1980
Biology of Bone Marrow Transplantation (2)
Membrane Transport and Neuroreceptors (1)
Control of Cellular Division and Development (1)
Animal Virus Genetics (2)
Mechanistic Studies of DNA Replication and Genetic Recombination (2)

1981
Immunoglobulin Idiotypes (2)
Initiation of DNA Replication (2)
Genetic Variation among Influenza Viruses (2)
Developmental Biology Using Purified Genes (2)
Differentiation and Function of Hematopoietic Cell Surfaces (1)
Mechanisms of Chemical Carcinogenesis (1)
Cellular Recognition (1)

1982
B and T Cell Tumors (2)
Interferon (2)
Rational Basis for Chemotherapy (1)
Gene Regulation (2)
Tumor Viruses and Differentiation (1)
Evolution of Hormone-Receptor Systems (1)

Publishers

(1) Alan R. Liss, Inc.
41 East 11th Street
New York, NY 10003

(2) Academic Press, Inc.
111 Fifth Avenue
New York, NY 10003

(3) W.A. Benjamin, Inc.
2725 Sand Hill Road
Menlo Park, CA 94025

(4) Plenum Publishing Corp.
227 W. 17th Street
New York, NY 10011

Pages 1–91 of this volume are reprinted from the Journal of Cellular Biochemistry, Volumes 26 and 27. The Journal is the only appropriate literature citation for the articles printed on these pages. The page numbers in the table of contents, contributors list, and index of this volume correspond to the page numbers at the foot of these pages.

The table of contents does not necessarily follow the pattern of the plenary sessions. Instead, it reflects the thrust of the meeting as it evolved from the combination of plenary sessions, poster sessions, and workshops, culminating in the final collection of invited papers, submitted papers, and workshop summaries. The order in which articles appear in this volume does not follow the order of citation in the table of contents. Many of the articles in this volume were published in the Journal of Cellular Biochemistry, and they are reprinted here. These articles appear in the order in which they were accepted for publication and then published in the Journal. They are followed by papers which were submitted solely for publication in the proceedings.

Contributors

Michael A. Alejos, Department of Cell Biology, University of Texas Health Science Center, Dallas, TX 75235 **[55]**

Ruth Hogue Angeletti, Laboratory of Neuropathology, Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia PA 19104 **[31]**

Hirohiko Aoyama, Institut d'Embryologie du CNRS et du College de France, 94130 Nogent sur Marne, France **[39]**

Rene Arentzen, Central Research & Development Department, E.I. du Pont de Nemours and Company, Wilmington, DE 19898 **[65]**

Frank Baldino, Jr., Central Research & Development Department, E.I. du Pont de Nemours and Company, Wilmington, DE 19898 **[65]**

R.L. Barchi, Institute of Neurological Sciences and the Department of Neurology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104 **[9]**

Martin W. Berchtold, Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030; present address: Universitat Zurich-Irchel, Institut fur Pharmakologie und Biochemie, Arbeitsgruppe Biochemie, Winterhurerstrasse 190, CH-8057 Zurich, Switzerland **[169]**

Mordechai Bodner, Departments of Hormone Research and Organic Chemistry, The Weizmann Institute of Science, Rehovot, Israel **[21]**

Daniel F. Bowen-Pope, Department of Pathology, SM-30, University of Washington, Seattle, WA 98195 **[145]**

Martin Carden, Laboratory of Neuropathology, Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104 **[31]**

Michael Comb, Department of Chemistry, University of Oregon, Eugene, OR 97403; present address: Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114 **[179]**

Martha Constantine-Paton, Department of Biology, Princeton University, Princeton, NJ 08544; present address: Biology Department, Yale University, New Haven, CT 06511 **[151]**

Erica J. Cox, Department of Cell Biology, University of Texas Health Science Center, Dallas, TX 75235 **[55]**

Leonard G. Davis, Central Research & Development Department, E.I. du Pont de Nemours and Company, Wilmington, DE 19898 **[65]**

James Douglass, Department of Chemistry, University of Oregon, Eugene, Oregon 97403 **[179]**

Jean Loup Duband, Institut d'Embryologie du CNRS et du College de France, 94130 Nogent sur Marne, France **[39]**

Mati Fridkin, Department of Organic Chemistry, The Weizmann Institute of Science, Rehovot, Israel **[21]**

The number in brackets is the opening page number of the contributor's article.

Jonathan A. Fuller, Department of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794 **[127]**

R.E. Furman, Institute of Neurological Sciences and the Department of Neurology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104 **[9]**

Joel M. Gottesfeld, Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, CA 92037 **[93]**

Illana Gozes, Department of Hormone Research, The Weizmann Institute of Science, Rehovot, Israel **[21]**

Karen F. Greif, Department of Biology, Bryn Mawr College, Bryn Mawr, Pennsylvania, PA 19010 **[1]**

W. Sue T. Griffin, Department of Cell Biology, University of Texas Health Science Center, Dallas, TX 75235 **[55]**

Ashley T. Haase, Infectious Disease Section, Veterans Administration Medical Center, San Francisco, CA 94121; present address: Department of Microbiology, University of Minnesota, Minneapolis, MN 55455 **[121]**

Edward Herbert, Department of Chemistry, University of Oregon, Eugene, OR 97403 **[179]**

Gerald A. Higgins, Central Research & Development Department, E.I. du Pont de Nemours and Company, Wilmington, DE 19898 **[65]**

James E. Krause, Department of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794; present address: Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110 **[127]**

Norbert E. Kremer, Department of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794 **[127]**

J.F. Kuo, Department of Pharmacology, Emory University School of Medicine, Atlanta, GA 30322 **[101]**

Raymond J. Lasek, Department of Developmental Genetics and Anatomy, Case Western Reserve University School of Medicine, Cleveland, OH 44106 **[135]**

Elias Lazarides, Division of Biology, California Institute of Technology, Pasadena, CA 91125 **[73]**

Virginia M.-Y. Lee, Laboratory of Neuropathology, Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104 **[31]**

Yuan Lin, Central Research & Development Department, E.I. du Pont de Nemours and Company, Wilmington, DE 19898 **[65]**

Dane Liston, Department of Chemistry, University of Oregon, Eugene, OR 97403 **[179]**

Robert W. Manning, Central Research & Development Department, E.I. du Pont de Nemours and Company, Wilmington, DE 19898 **[65]**

Mitchel Martin, Department of Chemistry, University of Oregon, Eugene, OR 97403 **[179]**

Jeffrey F. McKelvy, Department of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794 **[127]**

Anthony R. Means, Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030 **[169]**

Robert J. Milner, Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, CA 92037 **[93]**

Marcelle R. Morrison, Department of Neurology, University of Texas Health Science Center, Dallas, TX 75235 **[55]**

W. James Nelson, Division of Biology, California Institute of Technology, Pasadena, CA 91125 **[73]**

Monica M. Oblinger, Department of Developmental Genetics and Anatomy, Case Western Reserve University School of Medicine, Cleveland, OH 44106; present address: Department of Biological Chemistry and Structure, The Chicago Medical School, North Chicago, IL 60064 **[135]**

Paul O'Lague, Biology Department, University of California, Los Angeles, CA 90024 **[xvii]**

Thomas J. Poole, Institut d'Embryologie du CNRS et du College de France, 94130 Nogent sur Marne, France **[39]**

John A. Putkey, Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030 **[169]**

De-Fang Qi, Department of Pharmacology, Emory University School of Medicine, Atlanta, GA 30322; present address: Shanghai Institute of Biochemistry, Chinese Academy of Sciences, Shanghai, People's Republic of China **[101]**

Thomas A. Reh, Department of Biology, Princeton University, Princeton, NJ 08544; present address: Department of Medical Physiology, University of Calgary, Alberta T2N 4N1 Canada **[151]**

Wanda Reynolds, Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, CA 92037 **[93]**

Michael A. Rogawski, Laboratory of Neurophysiology, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, MD 20205; present address: Department of Neurology, The John Hopkins Hospital, Baltimore, MD 21205 **[113]**

Russell Ross, Department of Pathology, SM-30, University of Washington, Seattle, WA 98195 **[145]**

William W. Schlaepfer, Laboratory of Neuropathology, Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104 **[31]**

Audrey Seasholtz, Department of Chemistry, University of Oregon, Eugene, OR 97403 **[179]**

Ronald A. Seifert, Department of Pathology, SM-30, University of Washington, Seattle, WA 98195 **[145]**

Yael Shani, Department of Hormone Research, The Weizmann Institute of Science, Rehovot, Israel **[21]**

Rosalia C.M. Simmen, Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030 **[169]**

Kim D. Stewart, Department of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794 **[127]**

J. Gregor Sutcliffe, Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, CA 92037 **[93]**

J.C. Tanaka, Institute of Neurological Sciences and the Department of Neurology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104 **[9]**

Jean Paul Thiery, Institut d'Embryologie du CNRS et du College de France, 94130 Nogent sur Marne, France **[39]**

Holly I. Trenchard, Department of Biology, Bryn Mawr College, Bryn Mawr, Pennsylvania PA 19010 **[1]**

John Q. Trojanowski, Laboratory of Neuropathology, Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104 **[31]**

Gordon C. Tucker, Institut d'Embryologie du CNRS et du College de France, 94130 Nogent sur Marne, France **[39]**

R. Scott Turner, Department of Pharmacology, Emory University School of Medicine, Atlanta, GA 30322 **[101]**

Michel Vincent, Institut d'Embryologie du CNRS et du College de France, 94130 Nogent sur Marne, France; present address: Département de Génétique Moléculaire et Humaine, Centre Hospitalier de l'Université Laval, Saint Foy, G1V 4G2 Canada **[39]**

Jeffrey D. White, Department of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794 **[127]**

Betty Wolfson, Central Research & Development Department, E.I. du Pont de Nemours and Company, Wilmington, DE 19898 **[65]**

Preface

One of the fastest growing areas of modern biology is the molecular biology of gene structure and function. This growth has its origins in techniques that now allow isolation, duplication, and amplification of genetic information and fairly rapid sequencing of long DNA stretches. The amount of information accrued with the help of these techniques over the last five years is nothing short of staggering. On the other hand, one of the last areas likely to be unravelled is that associated with uncovering molecular rules governing the development and organization of neurons into hierarchical synaptic networks responsible for mediating animal behavior and its modification with experience. Topics encompassing both these areas were the focus of a UCLA Symposium held in Keystone, Colorado in April of 1984. This meeting brought together an international group of neurobiologists and molecular biologists, all of whom willingly participated in informal exchange of information. From the intensity of exchange, we felt the meeting was a great success.

The papers in this volume are representative of the many topics discussed at the meeting, which included cytoskeletal architecture, receptors and growth factors, gene expression and multigene families, membrane channels, intracellular messengers, neuropeptides and transmitter plasticity, and organizational principles in the development of neuronal networks. The present volume is divided into four sections. The first contains papers on cell adhesion, intercellular and virus-cell interactions in the nervous system. Recent observations are presented on how migratory and adhesive properties govern the formation of peripheral ganglia, on transsynaptic regulation of extracellular matrix components, and on how synaptic interactions affect retinotectal maps. This section concludes with an important paper on viruses and the nervous system in which recent autoradiographic techniques of general interest to both molecular biologists and neurobiologists are presented. The second section has articles dealing with recent observations on cytoskeletal architecture. This highly focused section contains papers describing the use of monoclonal antibodies directed against specific cytoskeletal proteins, in situ hybridization techniques for the mRNA of cytoskeletal elements, evidence for membrane-cytoskeletal protein interactions, and regulation of neuronal cytoskeletal networks. The third section consists of papers on neuropeptides and growth factors and describes recent biochemical observations on neuropeptide regulation and growth factor functions. In addition, the papers give a glimpse at how the most up-to-date molecular biological techniques such as, for example, in situ hybridization, cDNA cloning, use of synthetic oligonucleotide probes, and DNA sequencing is being brought to bear on problems of brain chemistry. The fourth section concludes the volume with papers on intracellular messengers and ion channels. Problems dealt with here include molecular

characteristics and functional reconstitution of excitable channels, electrophysiology of single channels, and phospholipid/Ca^{++}-dependent protein phosphorylation. The final paper in this section summarizes new molecular biological studies on calmodulin gene cloning, genes of great interest to neurobiologists because of their role in neuronal functioning. This volume should be useful for anyone interested in learning about some new directions in the study of the nervous system.

The program of this symposium was created with the advice and active participation of an Advisory Committee consisting of Richard Horn, Jim Patrick, Lewis Reichardt, Richard Scheller, and Greg Sutcliff. After deliberations by phone, we met at the Jerry Lewis Neuromuscular Research Center for a day of lively discussion in which the final details of program organization were thrashed out. After having made difficult decisions as to who should be invited, we then were faced with finding resources to help them come. Major gifts from Johnson and Johnson, McNeil Pharmaceutical Company, Sandoz, Ltd., E.I. du Pont de Nemours & Company, and Hoffman-La Roche, Inc. provided supplementary funding for travel and subsistence support for those invited meeting participants. All of us who attended are particularly grateful to Betty Handy for her efforts at the meeting which assured smooth operations, and to Sandy Malone and Hank Harwood whose work prior to the meeting made my part in it a pleasure rather than a chore.

Finally, I would like to thank Lynn Ianni, who coordinated editorial review by the Journal of Cellular Biochemistry for many of these articles, and who contributed the eleventh hour energy which helped close out this volume.

Paul O'Lague

Contents

Journal of Cellular Biochemistry 26:127–133 (1984)
Neurobiology: Molecular Biological Approaches to
Understanding Neuronal Function and Development 1–7

Evidence for Transsynaptic Regulation of Neuronal Cell Surface Heparan Sulfate Proteoglycan in Developing Rat Superior Cervical Ganglion

Karen F. Greif and Holly I. Trenchard

Department of Biology, Bryn Mawr College, Bryn Mawr, Pennsylvania 19010

The effect of neonatal deafferentation on the expression of a neuronal cell surface heparan sulfate proteoglycan (HeS-PG) was investigated in the developing rat superior cervical ganglion. Two monoclonal antibodies, one directed against the core protein of HeS-PG, and one to a determinant associated with a heparan sulfate side-chain, were used to monitor postnatal increases of HeS-PG by radioimmunoassay. Following neonatal deafferentation by section of the cervical sympathetic trunk, total protein per ganglion was slightly reduced at survival times of 7, 14, and 30 days. Expression of the core protein determinant on HeS-PG was not altered in deafferented ganglia. In contrast, levels of side-chain determinant were significantly reduced at 14 and 30 days. These results suggest that processing of HeS-PG side-chains by principal ganglionic neurons is partially regulated by transsynaptic influences during development. Transsynaptic regulation of neuronal development may be a more general process than was believed previously, with effects not limited to molecules associated with synaptic development.

Key words: radioimmunoassay, superior cervical ganglion, heparan sulfate, transsynaptic regulation

The rat superior cervical ganglion (SCG) has been used as a model system for studies of normal development and of the role of innervation in the regulation of development. Biochemical maturation of ganglionic neurons has been carried out by assay of tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine biosynthesis [1,2]. Estimates of the progress of synaptogenesis by cholinergic preganglionic neurons have been carried out by assay of choline acetyltransferase (CAT) [1,3,4]. Morphological assessment of principal ganglionic neurons and of developing synapses has been carried out by electron microscopic analysis [1,5,6]. These studies have shown that the SCG completes its development postnatally, with increases in TH levels [1], and increases in ganglionic cell size. Recognizable synapses are rare in the SCG in neonatal animals, with synaptogenesis occurring most rapidly during the first two postnatal weeks [1,3,6].

Received April 19, 1984; accepted June 18, 1984.

Because of the ease of manipulation of its presynaptic input, the SCG has been used as a model in which to study transsynaptic effects of presynaptic input. Section of the cervical sympathetic trunk (CST) in neonatal rats affects both the pattern of neuronal maturation and that of synaptogenesis [1–3,6–8]. Isolation of the SCG from developing presynaptic input at birth results in reduction in the postnatal increase in TH levels and reduces cell division and growth [1–3]. The development of postsynaptic specializations associated with synaptogenesis also does not occur [6]. Since denervation can be mimicked by treatment with ganglionic blockers such as chlorisondamine and pempidine [4], it has been hypothesized that presynaptic input exerts its developmental effects via direct synaptic activity. However, additional trophic influences may contribute to postsynaptic changes associated with denervation [9].

If reinnervation is permitted, up to 50% of normal levels of synapses can be recovered by 60 days [8]. However, TH levels do not recover [10]. In an apparent paradoxical result, assay by high-performance liquid chromatography (HPLC) of catecholamine levels present in both deafferented and reinnervated ganglia do not differ from those measured in control ganglia [10]. Thus, despite persisting reductions in TH levels, catecholamine pools remain at control levels.

Recently, monoclonal antibodies have been used to monitor the development of a neuronal cell surface heparan sulfate proteoglycan (HeS-PG) in the SCG [11]. Antibodies directed against two different determinants on HeS-PG, one associated with the core protein and one with a heparan sulfate side chain, were used to assess neuronal maturation. Quantitation of antigen levels by radioimmunoassay (RIA) indicated that these antigens increased significantly during postnatal development, with the most dramatic increases occurring during the second postnatal week. No significant redistribution of antigen associated with development was observed. These antigens were of interest because of the association of HeS-PG with a factor that induces neurite outgrowth in vitro [12,13]. The antigen can be used as a marker for neuronal surface to monitor neurite outgrowth and increases in cell diameter. Alterations in expression may be associated with the regulation of neurite outgrowth.

Because HeS-PG may represent a molecule associated with more general characteristics of development than neurotransmitter synthetic enzymes, we were interested in examining whether the expression of determinants on both core protein and side chains is regulated by presynaptic input. If the levels of these molecules are also reduced by neonatal deafferentation, it may be possible to conclude that transsynaptic regulation of development is a factor not limited to the control of molecules directly associated with synaptic transmission. A preliminary report of a portion of this work has been published [14].

METHODS

Reagents

Monoclonal antibodies directed against HeS-PG were obtained from Drs. L.F. Reichardt and W.D. Matthew. Monoclonal antibody PG 3 recognizes an antigenic determinant associated with a heparan sulfate side chain, and PG 22 recognizes the core protein or an N-linked carbohydrate associated with it. These antibodies were used either diluted directly from culture supernatants or from ammonium sulfate precipitations of ascites fluid. Previous studies have indicated that no differences in

the properties of antibodies derived from these sources can be observed [11]. ^{125}I-goat-anti-mouse-IgG (Fab′ fragment) or ^{125}I-sheep-anti-mouse-IgG (whole molecule) were obtained from Amersham.

Experimental Animals and Surgical Procedures

Sprague-Dawley rats were used throughout for these experiments. For neonatal section of the CST, published procedures were followed [6,8]. Litters of pups aged 1–4 days were removed from their mothers. The sex of each pup was determined and equal numbers of males and females were selected for experimental and control groups. Since unilateral deafferentation of the SCG can cause compensatory changes in the contralateral ganglion (Smolen, personal communication), all surgery was carrried out bilaterally. Control rats were littermates of experimental animals in all cases. Experimental animals were anesthesized with methoxyfluorane in air. The skin over the throat was opened and the carotid artery was visualized. The SCG was located beneath the carotid artery and the CST severed at the base of the ganglion. A 2–3-mm section of the CST was removed to prevent reinnervation. Section of the CST was carried out bilaterally. The skin was sealed with collodion dissolved in acetone. Pups were kept warm until wide-awake and were returned as a group, along with control animals, to their mother. Mortality was less than 20% for 7-day survival periods, with somewhat poorer survival rates with longer survival periods.

Surgery on adult female rats was carried out using chloral hydrate (35 mg/kg, 7% solution) as anesthetic. Bilateral section of the CST was carried out in a similar fashion. The fascia surrounding the salivary glands and the overlying skin were sutured separately using 4-0 silk. Rats were given intramuscular injections of 100,000 units of Bicillin to reduce postsurgical respiratory infections. Mortality was less than 5% using prophylactic doses of antibiotic.

Quantitation of Antigen

Assay of antigen levels was carried out according to previously developed methods [11]. Ganglia from experimental animals were pooled to provide sufficient tissue for assay. Control ganglia were treated similarly. Desheathed ganglia were homogenized in 5 mM Tris-HCl, pH 8.1, in the presence of a protease inhibitor, phenylmethylsulfonylfluoride. Protein levels were determined by Amido-Schwarz assay. The total protein per ganglion was determined by correcting this value for total volume of homogenate and the number of ganglia pooled. Twelve dilutions of whole tissue homogenate were prepared (from 1:1 to 1:10,000) and aliquots were incubated with equal volumes of antibody at limiting dilution. After 18–24 hr, bound antibody was precipitated by centrifugation at 100,000g in an Airfuge. The amount of antibody remaining in the supernatant was determined by a standard solid-phase RIA [15], using a crude preparation of rat brain synaptosomes as the antigen. 50% inhibition points (I-50) were determined graphically, and standardized to 4 mg/ml initial concentration of protein. These values were further corrected for variations in protein content per ganglion, to permit comparison between experimental and control groups at each age examined, according to I-50 (corrected) = I-50 × [total protein (control ganglia)/ total protein (experimental ganglia)]. Control results were standardized to a value of 1.0; results from experimental groups were calculated as antigen levels relative to control levels (relative specific activity, RSA). All homogenates were assayed at least twice.