METHODS IN MOLECULAR BIOLOGY™

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Neuropeptides

Methods and Protocols

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Humana Press

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ISSN 1064-3745 e-ISSN 1940-6029
ISBN 978-1-61779-309-7 e-ISBN 978-1-61779-310-3
DOI 10.1007/978-1-61779-310-3
Springer New York Dordrecht Heidelberg London

Library of Congress Control Number: 2011936011

Printed on acid-free paper

Humana Press is part of Springer Science+Business Media (www.springer.com)

Preface

The term *neuropeptide* was originally coined to indicate small protein molecules that are contained in neurons. In the late 1970s and the 1980s of the last century, several tens of neuropeptides were localized by immunocytochemistry to discrete cell populations of the central and peripheral nervous system, and the concept of *chemical neuroanatomy*, originally developed by Tomas Hökfelt and coworkers, entered the scene of neurobiology. Since then, the field of neuropeptide biology has dramatically widened, and today the ultimate frontiers in neuropeptide research lie in the development of pharmacologically active compounds that are capable of crossing the blood–brain barrier to exert their biological role(s) in vivo and in the construction of genetic vectors to be employed in gene therapy.

This book represents a readily reproducible collection of established and emerging techniques for neuropeptide research. Such a collection is preceded by a general introductory chapter (Chapter 1) that discusses a series of new concepts leading to a broader neuropeptide definition in light of the huge amount of data accumulated after more than half a century of neuropeptide research.

The methods presented include immunocytochemical localization, biochemical characterization, functional analysis, development and production of genetic probes, and the design of neuropeptide derivatives for cellular neurobiology as well as the potential therapeutic applications.

As a general indication to the readers, Chapters 2–10 are focused on a series of techniques for localization studies. They cover a broad range of protocols, such as the immunocytochemical detection of neuropeptides in nonmammalian vertebrates together with a detailed description of procedures for anesthesia and tissue preparations in these species (Chapter 2); the combined neuropeptide/receptor localization at the light and transmission electron microscope for connectivity studies (Chapter 3); the analysis of neuropeptide genes' transcription by localization of pre-mRNA (Chapter 6) or mRNA/microRNA with in situ hybridization (Chapter 4), in situ PCR (Chapter 5), and laser capture/microdissection (Chapter 7); the visualization in vivo of neuropeptide secretion (Chapter 8) and translocation across the plasma membrane (Chapter 9); and the functional analysis of neuropeptide interactions in vitro with cells of the immune system (Chapter 10).

Chapter 11 describes a series of electrophysiological protocols for functional studies in vitro and in vivo.

Chapters 12–19 are devoted to biochemical/molecular biology techniques, ranging from radioimmunoassay (Chapter 12) to neuropeptidomics employing reverse-phase HPLC (Chapter 13) or mass spectrometry (Chapter 14), RNA analysis by suppression subtractive hybridization (Chapter 15), determination of neuropeptide release in vivo by microdialysis (Chapter 16) or antibody microprobes (Chapter 17), and measurement of neuropeptidases (Chapter 18) and neuropeptide autoantibody levels (Chapter 19) in biological fluids.

Chapters 20–24 deal with a number of techniques developed to optimize neuropeptide administration to central neurons or to interfere with biological effects in vivo. These procedures include the intranasal delivery of neuropeptides (Chapter 20), the development of

neuropeptide pro-drugs (Chapter 21), the use of phosphorothioate oligodeoxynucleotides that are capable of crossing the blood–brain barrier to knock down neuropeptides in the CNS (Chapter 22), the development of liposome-encapsulated neuropeptides for assessing the chronic actions of physiologically short-lived molecules (Chapter 23), the construction of recombinant adeno-associated viral vectors that can be used to locally or systemically enhance or silence neuropeptide gene expression (Chapter 24).

Finally, Chapter 25 describes a calcium mobilization assay in mammalian cells to identify novel G-protein-coupled receptor family members that transduce the neuropeptide signals.

All scientists who have excellently contributed to this book have a direct experience in one or more fields of neuropeptide research. I am very much indebted to all of them for their successful effort in emphasizing the description of the more common pitfalls in the techniques that they have described and of the hints to reduce the possibility of failure for beginners.

The collection of protocols that forms this book is surely not exhaustive of the wide range of approaches that today can be employed in top level neuropeptide research. Yet it is intended for a large audience of scientists, including histologists, biochemists, cellular and molecular biologists, and electrophysiologists that are currently active in the field or are willing to enter such an exciting and still expanding area of neurobiology.

Grugliasco, TO, Italy ***Adalberto Merighi***

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In molecular biology, ligation refers to the joining of two DNA fragments through the formation of a phosphodiester bond. An enzyme known as a ligase catalyzes the ligation reaction. In the cell, ligases repair single and double strand breaks that occur during DNA replication.Â Critical aspects of ligation reactions are discussed, such as how the length of a sticky end overhang affects the reaction temperature and how the ratio of DNA insert to vector should be tailored to prevent self-ligation. Molecular tools that assist with ligations like the Klenow Fragment and shrimp alkaline phosphatase (SAP) are mentioned, and applications , such as proximity ligations and the addition of linkers to fragments for sequencing are also presented.â€¦ Basic Methods in Cellular and Molecular Biology. Macrophages and dendritic cells. Methods and protocols. Preface. Reiner NE. PMID: 19422172. DOI: 10.1007/978-1-59745-396-7.Â Molecular Biology/methods*. Phagocytosis. Receptors, Cell Surface/metabolism. Substance. Receptors, Cell Surface. LinkOut - more resources. Full Text Sources. In â€œNeuropeptides: Methods and Protocols, Methods in Molecular Biologyâ€. Chapter Â· July 2011 with 3 Reads. How we measure 'reads'.Â A method was developed that allows the analysis of neuropeptides and monoamines in a single tissue section by the application of the unlabeled antibody method for peptide staining to tissue sections freeze-dried for formaldehyde-induced monoamine histofluorescence. The hypothalamic magnocellular system of male albino rats served as a model for this study; neurons were stained with