METHODS IN MOLECULAR BIOLOGY™

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Neuropeptides

Methods and Protocols

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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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Researchers in molecular biology use specific techniques native to molecular biology but increasingly combine these with techniques and ideas from genetics and biochemistry. There is not a defined line between these disciplines. The figure to the right is a schematic that depicts one possible view of the relationships between the fields:[3].Â Molecular biology is the study of molecular underpinnings of the processes of replication, transcription, translation, and cell function. The central dogma of molecular biology where genetic material is transcribed into RNA and then translated into protein, despite being oversimplified, still provides a good starting point for understanding the field.Â RNA methods and protocols. New York, N.Y.: Humana Press. pp. 87â€“105. Molecular Cytogenetics: Methods and Protocols, edited by Yao-Shan Fan, 2002 203. In Situ Detection of DNA Damage: Methods and Protocols, edited by Vladimir V. Didenko, 2002 202. Thyroid Hormone Receptors: Methods and Protocols, edited by Aria Baniahmad, 2002 201. Combinatorial Library Methods and Protocols, edited by Lisa B. English, 2002 200.Â Neuropeptide Y Protocols , edited by Ambikaipakan Balasubramaniam, 2000 152. DNA Repair Protocols: Prokaryotic Systems, edited by Patrick Vaughan, 2000. Methods in molecular biology. Tm. PCR Cloning Protocols Second Edition Edited by. Neuropeptides: Methods and Protocols presents a readily reproducible collection of established and emerging techniques for neuropeptide research as contributed by expert researchers in the field. The detailed methods presented cover subjects such as 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. Written in the highly successful Methods in Molecular Biologyâ„¢ series format, chapters include intro Start by marking â€œMethods in Molecular Biology, Volume 73: Neuropeptide Protocolsâ€ as Want to Read: Want to Read savingâ€¦ Want to Read.Â Leading scientists offer an unprecedented suite of master protocols for analyzing neuropeptide structure and function using optimized classic methods and the latest state-of-the-art techniques. This comprehensive collection includes valuable techniques for extracting and purifying neuropeptides from biological sources, for their sequence determination by Edman degradation Leading scientists offer an unprecedented suite of master protocols for analyzing neuropeptide structure and function using optimized classic methods and the latest state-of-the-art techniques.