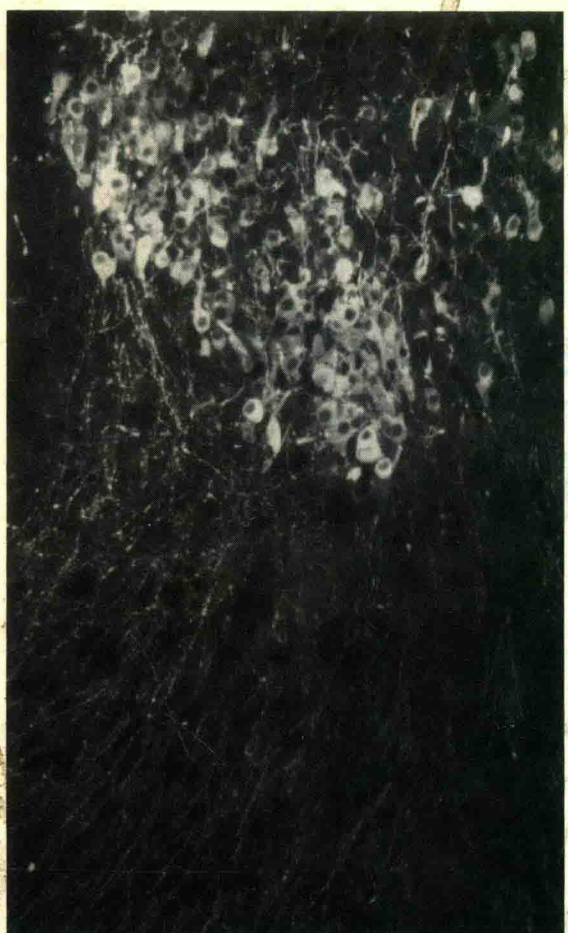
THE ROLE OF PEPTIDES IN NEURONAL FUNCTION



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PREFACE

This volume should be considered a progress report on recent activities in a rapidly expanding area of research focussed on the role of peptides in neuronal function. Interest in this area has increased so dramatically that fifteen separate symposia were held and two journals on the neurobiology of peptides were started during 1979 and 1980. Although this collection of papers is not a wholly comprehensive account of the field, the contents of the book aim to illuminate a significant area of neuroscientific endeavor by reviewing the principal strategies that have evolved and observations on peptides which have thus far received the most extensive, multidisciplinary study.

Peptides are chains of amino acids linked together by peptide bonds. Peptides may thus be considered as proteins of short chain length. The first neuronal peptides to be characterized were extracted from the nervous system about thirty years ago. They came from a part of the brain which releases its peptide products into the general circulation and were named according to their primary actions on peripheral target tissues. The dual functions discovered for one of these peptides led to two names for one substance: antiduretic hormone and vasopressin. The next peptides to be extensively investigated were extracted from a part of the brain which releases its peptides into the pituitary portal circulation for the purpose of regulating the endocrine activities of the pituitary. These peptides were then named according to their characteristic actions on target cells as luteinizing hormone releasing hormone, thryotropin releasing hormone, somatostatin, *et cetera*. These initial observations showed that specific peptide substances

could mediate hormonal signals in both general and pituitary portal circulations.

The past decade of research on peptides has provided convincing evidence that neuronal peptides also play important roles within the nervous system. A variety of peptides and peptide receptors have now been found throughout the nervous system. When applied to the nervous system in a pharmacologic manner these substances produce a multiplicity of diverse and dramatic effects. For example, all the components of the renin-angiotensin system, which has a long established peripheral role in the regulation of salt and water balance, now appear to be present in the central nervous system (see chapter by Phillips). Pharmacologic application of renin or angiotensin elicits drinking behavior, a release of antiduretic hormone and an increase in blood pressure. Luteinizing hormone releasing hormone, initially shown to have a role in the pituitary-reproductive system axis, is now implicated in some aspects of sexual behavior generated by the central nervous sytem (see chapter by Moss and Dudley) and in communication through sympathetic ganglia (see chapter by Barker, and colleagues). Substance P, first described for its effects in gastronintestinal and salivary functions, appears to mediate pain sensation and local vasodilatory reflexes in response to pain (see chapter by Phillis). And recent evidence suggests that the cholecystokinin octapeptide may be important in eating behavior (see chapter by Snyder and co-workers).

From the foregoing it is apparent that peptide substances mediate signals from the nervous system to target cells in the periphery and between cells within the nervous system. Another significant development in this area of research comes from the observation that one type of peptide receptor is a site which binds a clinically important class of drugs, the opiates. This suggests that some of the pharmacological actions of the opiates are mediated through engagement of receptors for endogenous

peptide ligands (see chapter by Kosterlitz). It is possible that other classes of drugs owe their pharmacologic actions to engagement of receptors for other endogenous ligands. Physiological roles for these "opioid" peptides and for all the other peptides discovered thus far have yet to be specificied owing to the natural complexity of the tissue in which they are found. Undoubtedly many years of effort will be required to understand why peptides, in addition to non-peptide neuronal substances which have previously been identified, have evolved and been preserved throughout evolution.

The first part of this book is concerned with the basic strategies that have been applied to the study of neuronal peptides. These include 1) investigations into the localization of peptides at the light and electron microscopic levels, 2) studies on the synthesis, transport, and release of peptides, and 3) research on the physiology of peptidergic neurons and on the electrophysiological description of peptide actions at the membrane level. The second half of the volume summarizes observations on those peptides where several of the aforementioned strategies have been applied. Although the importance of these peptides and their roles in neuronal function cannot yet be viewed with sufficient perspective to permit more than an initial understanding, the material presented here should provide an historical basis for considering future research applications in this area.

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IMMUNOHISTOCHEMICAL TECHNIQUES FOR THE ANALYSIS OF PEPTIDERGIC NEURONS

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I. INTRODUCTION

Neurohistologists have long been concerned with describing the cellular architecture of the nervous system. Our present understanding of neuronal morphology and pathways has, in general, relied upon histological methods that do not differentiate between neurons on the basis of the molecules they utilize for interneuronal communication. However, within the last two decades new techniques have been developed, and older methods adapted for histochemical studies of specific neurotransmitters within histological sections.

Neuropeptides are a recently discovered class of agents which may be involved in interneuronal communication. With advances in peptide isolation and characterization techniques, as well as greater capabilities to produce specific antisera to small peptides, several immunohistochemical methods have emerged as useful tools to study the distribution of peptides in the nervous system.

Just one decade ago, the first immunohistochemical localization of a substance related to neurotransmission was reported. In this pioneering work, Geffen and colleagues (1) reported the localization of dopamine-β-hydroxylase by immunofluorescence. Since then many neuroeffector substances have been isolated and chemically characterized, and a number of new modifications of immunohistochemical techniques have been reported. In this chapter, an attempt will be made to critically review some immunohistochemical techniques most applicable to the study of neuropeptides.

II. LIGHT MICROSCOPIC TECHNIQUES

A. Immunohistochemical Detection Systems

Present methods for immunological localization of neuropeptides in tissue sections stem from the indirect immunofluorescent techniques developed by Coons (2, Fig. 1a). The technique is based on the ability of an antibody raised in response to immuno-

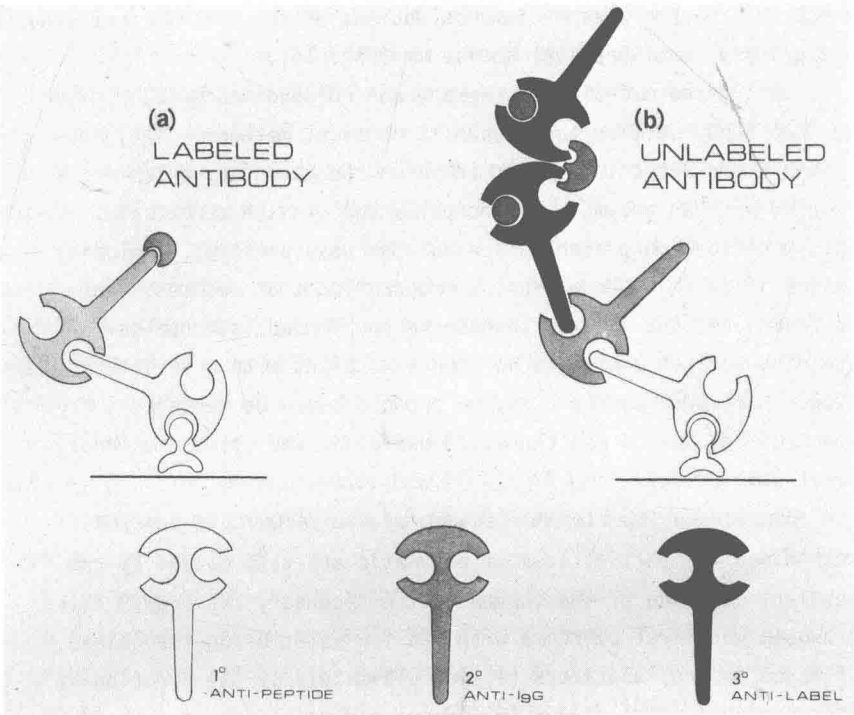

Fig. 1. Schematic of indirect immunohistochemical techniques. a) The labeled antibody method. The primary antibody (anti-peptide) is shown with one combining site bound to the exposed determinant of the immobilized peptide in the tissue section. The presence of this peptide-antibody complex is detected by the binding of a secondary antibody directed against immunoglobulins of the species providing the primary antibody. The secondary antibody is labeled with either a fluorchrome (2) or an enzyme (3) which can be histochemically revealed. b) The unlabeled antibody method. The presence of the peptide-antibody complex is revealed by a secondary, unlabeled antibody which links the primary antibody to a soluble complex of teriary antibody-enzyme [generally peroxidase anti-peroxidase (5)]. Again, the enzyme is revealed histochemically.

logic challenge by the peptide to bind to a determinant of the immobilized peptide in the tissue section. In the indirect immunofluorescent method the peptide-antibody complex is revealed by the subsequent binding of a fluorescein labeled secondary antibody