HANDBOOK OF CHEMICAL NEUROANATOMY

Edited by A. Björklund and T. Hökfelt

Volume 4:

GABA AND NEUROPEPTIDES IN THE CNS, PART I

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1985

ELSEVIER
Amsterdam – New York – Oxford

ISBN 0 444 90353 4
ISBN SERIES 0 444 90340 2

Library of Congress Cataloging-in-Publication Data
Main entry under title:

GABA and neuropeptides in the CNS.
(Handbook of chemical neuroanatomy ; v. 4, etc.)
Includes bibliographies and index.
1. Neuropeptides–Analysis. 2. GABA–Analysis.
3.Central nervous system–Anatomy. I. Björklund, Anders, 1945- . II. Hökfelt, Tomas. III. Series.
[DNLM: 1. Central Nervous System. 2. GABA. 3. Nerve Tissue Proteins. W1 HA51J v. 4 etc. / WL 104 G112]
QM451.H24 1983 vol. 4, etc. 616.8 s 85-20596
[QP552.N39] [599'.01'88]
ISBN 0-444-90353-4 (U.S. : pt. 1)

Published by:
Elsevier Science Publishers B.V.
P.O. Box 1126
1000 BC Amsterdam

Sole distributors for the USA and Canada:
Elsevier Science Publishing Co. Inc.
52 Vanderbilt Avenue
New York, NY 10017

Printed in The Netherlands by Casparie – Amsterdam

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Preface

The first neuroactive peptides to be structurally identified in the central nervous system were vasopressin and oxytocin (Du Vigneaud et al. 1953a,b). At that time, these peptides were viewed as hormones, and the so-called neurosecretory neurons (Bargmann and Scharrer 1951) which produce them were thought to be exceptional in that they secrete their products in an endocrine manner into the bloodstream. The current upsurge of interest in the role of small peptides as transmitter candidates or neuromodulatory compounds dates back to the early 1970's. Of great importance for this development was the discovery by Guillemin, Schally, Vale and their collaborators that the hypothalamic releasing and inhibitory hormones, originally postulated by Harris in 1955 – in terms of their chemistry – belong to the group of small peptides. Thus, thyrotropin releasing hormone (TRH) turned out to be a tripeptide (Bøler et al. 1969; Burgus et al. 1969), the luteinizing hormone releasing hormone (LHRH) a decapeptide (Amoss et al. 1971; Matsuo et al. 1971; Schally et al. 1971), and somatostatin, the growth hormone release inhibiting hormone, a tetradecapeptide (Brazeau et al. 1971).

At about the same time, Leeman and collaborators (Chang et al. 1971) were able to chemically characterize a compound, substance P, which was already discovered in 1931 by Von Euler and Gaddum. Substance P was also found to be a peptide, an undecapeptide. Using radioimmunoassay and immunohistochemistry, it was demonstrated that these peptides occur in neurons not only in the hypothalamus but also in widespread areas of the central nervous system, and in several cases also in peripheral neurons.

These discoveries drew attention to other peptide hormones that had been isolated and structurally characterized much earlier, for example cholecystokinin (CCK), first described by Mutt and Jorpes in 1968. In fact, the demonstration of gastrin/CCK-like immunoreactivity in the brain by Vanderhaeghen and coworkers (1975) showed that a polypeptide which so far had been associated only with endocrine functions in the periphery, could also be present in the central nervous system. These and many other findings were the basis for the growing interest in and formulation of the concept of the brain-gut axis.

During the seventies and eighties, numerous other neuronal peptides have been discovered. Snyder (1980), discussing a possible transmitter role of peptides in the central nervous system, predicted that their number may well be several hundred, and the development since then has supported that prediction. In fact, an important issue has been the realization that families of peptides may exist. One example is the dramatic progress in the field of opioid peptides, starting with the discovery by Hughes, Kosterlitz and collaborators (1975) of two pentapeptides, the enkephalins, with opioid activity in the brain. Thanks to the work of many groups, notably Eipper and Mains and the teams around Chrétien, Goldstein, Herbert and Numa, this research has resulted in less than a decade in a fairly complete knowledge of the precursor molecules not only of these two pentapeptides but also of two other opioid peptide precursors, and the description of a large number of biologically active cleavage products arising from these compounds (see Weber et al. 1983).

Furthermore, evidence has been presented that substance P is not the only tachykinin present in the mammalian nervous system, but that there are several compounds belonging to this peptide family. Thus, substance P and substance K are two peptides coded

for by the same gene; in addition, there are other tachykinins (Kangawa et al. 1983; Kimura et al. 1983; Nawa et al. 1983; Minamino et al. 1984; Tatemoto et al. 1985).

New powerful techniques have contributed to these advancements and to the explosive development in the peptide field. Thus, in addition to classical purification methods starting with huge amounts of tissue and purification steps monitored by parallel bioassay, there are now more chemically oriented methods, depending on for example, isolation of peptides containing C-terminal amide groups (Tatemoto and Mutt 1980). The DNA recombinant technology is now rapidly being introduced in the field of neurobiology. The discovery of a calcitonin gene related peptide (CGRP) (Amara et al. 1982; Rosenfeld et al. 1983) with powerful actions in the central and peripheral nervous systems represents a major breakthrough demonstrating the potential of this approach.

The *Neuropeptides* volumes of the *Handbook of Chemical Neuroanatomy* deal with the structural organization of neuropeptide-containing neuronal systems in the CNS, as revealed primarily by the immunohistochemical technique. In the second of the two *Neuropeptide* volumes so far planned, we have also included chapters dealing with peptide receptor localization based on the ligand binding techniques. Peptide distributions in peripheral tissues are covered in the *Peripheral Nervous System* volume of This Series.

The present volume also includes a major chapter by Mugnaini and Oertel on GABA neurons in the CNS as revealed by an antiserum raised against the GABA-synthesizing enzyme, glutamic acid decarboxylase (GAD). This chapter was originally planned for Volume 3, but due to the extensive work the authors had to invest in this chapter – reflecting the vast and complex distribution of the GABAergic system throughout all parts of the CNS – we chose to include it in the present volume. This chapter, which should be studied in conjunction with the chapters by Ottersen and Storm-Mathisen and by McGeer and collaborators in *Volume 3*, gives a unique overview of putative GABAergic neurons in the CNS and contains a wealth of previously unpublished material, including the first complete mapping of GAD in the rat CNS.

The term 'neuropeptide' is as yet poorly defined. The compounds included here share localizational features in that: (1) they are neuron-specific (although they occur in endocrine and paracrine types of cells as well); (2) they are characteristic for defined subsets of neurons, i.e. for some but not all neurons; and (3) they are likely to be secretory products of these neuronal subsets. Functionally, the compounds we now include among the neuropeptides may, on the other hand, turn out to be quite heterogeneous. For some of the most studied peptides, such as vasopressin, oxytocin and the hypophysiotrophic peptides, at least part of their biological properties (in particular their neurohormonal actions) are well known. But even so, the role of neuropeptides in neuronal communication is only beginning to be unraveled. This problem is particularly evident for individual members of the 'peptide families' such as the opioid peptide, substance P or the pancreatic polypeptide families mentioned above. It is possible, or perhaps even likely, that only some of these neuropeptides will turn out to possess neurotransmitter- or neuromodulator-type actions, while others may exert e.g. trophic, metabolic or vascular effects.

Regardless of their function, however, the neuropeptides have become important as chemical 'tags' for the study of neuronal circuitries. Thus, the neuropeptides and the neuropeptide families serve to label subsets of neurons that are, or are likely to be, functionally related, and sensitive and specific antibodies raised against the purified or synthetic compounds are today important tools for selective immunohistochemical visualization of such neuronal subsets. The availability of an increasing repertoire of neuropeptide antisera has in fact been of great importance for the rapid development of chemical neuroanatomy over the last decade.

The immunohistochemical visualization of peptide-containing neurons forms the basis of most chapters in the present volume. While this has proved to be a powerful approach, there are definite interpretational problems inherent to the technique which are important to bear in mind, viz. the shortcomings of the immunological recognition mechanisms which underlie the attachment of the antibodies to antigens in the tissue. Thus, most antibodies used recognize only a small part of a peptide molecule, and it is well known that such antigenic determinant parts may be shared by several different (although sometimes related) peptides. Specificity is therefore frequently a difficult problem in the interpretation of immunohistochemical pictures. In some fields, such as the opioid peptide or the pancreatic polypeptide family, this has been gradually sorted out through the combined use of antibodies directed against different parts of a molecule or against different related molecules. However, in other cases, e.g. the gastrin/CCK family of peptides or the tachykinin family of peptides, much work obviously remains in order to establish the diversity and identity of immunohistochemically visualized compounds. This elementary feature of antibody-based staining is the reason why most authors chose to characterize their observed material as (peptide)-like immunoreactivity. This is particularly warranted in microscopic material, where high concentrations of antiserum are used and where the tissue most often is exposed to fixatives (such as formaldehyde or glutaraldehyde) which can modify or significantly alter the chemical structure of the compounds under study.

Peptide research has a strong tradition in Sweden. Substance P, in a broad sense one of the best characterized neuronal peptides, was discovered by Von Euler and Gaddum in 1931. Börje Uvnäs and his collaborators have been working since the sixties on the physiology and pharmacology of gastrointestinal hormones. Viktor Mutt and his colleagues have for decades played a leading role in the work of isolating peptides from the gastrointestinal tract and, much based on their discoveries, an intense peptide research has been going on since the beginning of the seventies at Swedish Universities, especially at the Karolinska Institutet and in Lund.

The two volumes *'Neuropeptides in the CNS'* are dedicated to three of the pioneer Swedish scientists who have been of special importance to us and the development in this research area. They are Rolf Luft, Bengt Pernow and Viktor Mutt. Viktor Mutt, Professor of Biochemistry at Karolinska Institutet, has discovered more than a dozen peptides, mainly by isolation from the gastrointestinal tract. Among these peptides are cholecystokinin, vasoactive intestinal polypeptide (VIP), neuropeptide Y, peptide HI and peptide YY. Through his work and extreme generosity, laboratories not only in Sweden but all over the world have benefited greatly and obtained peptides for their research. Uncountable numbers of papers have been published based on peptides provided by Viktor Mutt and his associates.

Initiation of research in a new area is often the result of the insight and foresight of single individuals. Thus, the explosive development of peptide research in Sweden was much dependent on the initiative of Professor Bengt Pernow and Professor Rolf Luft. ... the beginning of the 1970's they were among the first to realize the interesting new opportunities that were opening up in the neuropeptide field. Bengt Pernow, Professor of Clinical Physiology at the Karolinska Hospital, wrote his now classical thesis on substance P (Pernow 1953) in the mid fifties, working in Ulf von Euler's laboratory. When Susan Leeman and her collaborators in 1971 sequenced substance P, Pernow realized the full potential of this discovery and initiated a new dynamic phase of substance P research which was then transmitted to many laboratories in Sweden, including our own. He recently summarized the progress in the substance P field in a review article

in *Pharmacological Reviews* (Pernow 1983). Rolf Luft was the first Professor of Endocrinology in Sweden, and already in the late forties he carried out work related to hypothalamic control of anterior pituitary hormone secretion. As a leading scientist in the field of diabetes, he became interested in somatostatin and initiated work at the Karolinska Institutet on the distribution and functional role of this peptide not only as a hypothalamic hormone but also, surprisingly, with regard to its involvement in the function of the endocrine pancreas (Luft et al. 1974).

It is an honour and pleasure to dedicate the neuropeptide volumes to these three pioneers and friends, who have been of great importance not only for our own research but for biomedical research in Sweden in general.

Lund and Stockholm in July 1985

ANDERS BJÖRKLUND TOMAS HÖKFELT

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