HANDBOOK OF CHEMICAL NEUROANATOMY

Edited by A. Björklund and T. Hökfelt

Volume 2:

CLASSICAL TRANSMITTERS IN THE CNS, PART I

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Preface

This second volume of the Handbook of Chemical Neuroanatomy has its primary focus on the catecholamine-producing neurons in the central nervous system. It is somewhat of a paradox that these systems, which were unknown to classical neuroanatomy and virtually undetectable with the classical neuroanatomical staining methods, today represent some of the very best known components of the brain with respect to the combined anatomical, physiological, chemical, pharmacological, behavioral and clinical knowledge. They have become a model for integrative transmitter-specific neurons in the brain with the capacity to influence and modulate a wide spectrum of CNS functions and behaviors.

The nine chapters of this volume represent a condensation of anatomical knowledge accumulated over three decades of study, from the first biochemical distributional studies on noradrenaline by Vogt in 1954, and on dopamine in 1958 (Carlsson et al. 1958; Bertler and Rosengren 1959), and the first histochemical demonstrations of catecholamine-containing neurons and terminal networks in the CNS by Carlsson, Falck and Hillarp in 1962.

The research groups assembled during the subsequent years by Nils-Åke Hillarp in Stockholm and by Bengt Falck in Lund came as close to a scientific 'school' as one could come in those days. This school has not only produced a whole generation of Swedish histochemists and neurobiologists, but it has also been intimately involved with the developments in catecholamine research over recent decades. To us the present volume is a tribute to this tradition.

The groundwork in Swedish monoamine histochemistry was laid by several of Hillarp's and Falck's respective pupils, and although not all of them appear as authors in the Handbook series, the chapters in this (as well as several other) volumes draw importantly on their works and discoveries. Much of this research was initiated in Hillarp's and Falck's laboratories as doctoral thesis works during the sixties, and collectively these M.D. theses give an impression of how Swedish monoamine histochemistry evolved.

One line of work concerned the anatomy and pharmacology of central monoaminergic systems, starting with Annica Dahlström and Kjell Fuxe's *Acta physiol. scand. Supplement*, published in 1964, and Fuxe's thesis (published in 1965), and subsequently followed up by Urban Ungerstedt (1971) and Olle Lindvall (1974). This work is fundamental to several of the chapters in the present volume. A second line of research focussed on the organization and dynamics of peripheral catecholamine neurons, represented by an impressive range of studies carried out by Christer Owman (1964), Torbjörn Malmfors (1965), Karl-Axel Norberg (1965), Annica Dahlström (1966), Bertil Hamberger (1967), Gösta Jonsson (1967) and Lars Olson (1970). A third major line of study developed as a result of a series of discoveries of monoaminergic mechanisms in endocrine and paracrine cell systems. This work, which gave the first clues to the functional similarities between neuronal and endocrine cell systems, as well as the co-localization of different active compounds in the same cells, was pioneered by Christer Owman (1964), Lennart Cegrell (1968), Rolf Håkanson (1970) and Frank Sundler (1973). The impact of these latter two research lines will be particularly evident in the forthcoming volume in this series on the Peripheral Nervous System (Volume 6).

Preface

The work to assemble present-day knowledge on the catecholaminergic systems has been particularly enjoyable and stimulating, not least because it so strongly ties back to the efforts and discoveries which we ourselves were fortunate to experience in the early parts of our research lives. One can never know exactly how Hillarp and Falck in the early 1960s envisioned the future developments in the field they initiated. Nevertheless, the progress reflected in the various chapters presented here may very well have satisfied even their wildest expectations. Elsewhere (Falck 1977), Bengt Falck has recalled the excitement over the perspectives opened by their early discoveries. This excitement they generously handed over to and shared with their young students and collaborators. Swedish neurohistochemistry still thrives on it. It is therefore an honour for us to dedicate this Volume to Nils-Åke Hillarp and Bengt Falck.

Lund and Stockholm in September 1984

ANDERS BJÖRKLUND TOMAS HÖKFELT

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CHAPTER I

General features of chemically identified neurons

FLOYD E. BLOOM

1. INTRODUCTION

A major question in neurobiology concerns the extent of information encoded by the chemical neurotransmitter used by a given neuron to transmit to its target cells. The multiple facets of this problem have been intensified by major recent advances in cellular neurobiology in three specific areas of work: neuronal connectivity, synaptic mechanisms, and neuronal transmitters. Innumerable interneuronal connections and new principles of connectivity have been revealed through the use of the sensitive and elegant new circuit tracing methods, revealing a far more detailed overall picture of the complexities and principles of brain organization. The new methods of electrophysiological analysis, particularly useful with the in vitro preparations, have offered a wide range of ionic conductance mechanisms, through which many neurotransmitters would appear to transmit their signals. Lastly, the modern methods of chemical analysis have provided an ever increasing list of new neurotransmitter molecules, distributed almost exclusively among three chemical categories: amino acids, monoamines, and neuropeptides. Almost all of these transmitters can now be localized sensitively with one or more cytochemical methods, with increased emphasis being placed on immunocytochemistry even for small non-peptides. This chapter will consider some possible underlying principles by which to approach the rich signalling capacity of the central nervous system, and particularly the question as to whether the specific chemical neurotransmitter used by a given neuron implies any further specification of any other structural or functional neuronal properties.

1.1. A BRIEF HISTORY OF NEUROTRANSMITTER RESEARCH STRATEGIES

When there were relatively few chemical substances available for examination as chemical transmitters, the major thrust of most research was aimed at establishing the identity of the transmitter for specific synaptic junctions: acetylcholine at the recurrent axonic synapses of spinal motoneurons on Renshaw interneurons (see Curtis and Johnston 1974; Johnston 1978; Krnjević 1974), and either amino acids, monoamines or peptides as possible mediators of recurrent inhibitions within the spinal cord (Werman 1972), olfactory bulb (Bloom et al. 1964; Salmoiraghi et al. 1964), hypothalamus (Barker et al. 1971; Dreifuss and Kelly 1972; Moss et al. 1972; Poulain and Wakerley 1982; Renaud 1976), cerebellum (see Johnston 1978; Krnjević 1974) and hippocampus (see Johnston 1978; Krnjević 1974).

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The rules for such work were relatively straightforward, and electrophysiologically based (see Bloom 1974). First, evidence was required to document that the transmitter candidate was 'present' (i.e., neurochemically detectable) in a region of the CNS in which the synaptic connection was to be studied. The synaptic target cell was then tested with extracellular recordings of discharge rates, and intracellular recordings of transmembrane changes in potential or impedance, and the effects of the transmitter candidate were compared with the effects of selective activation of the synaptic pathway. Qualitative similarities between the two tests were the starting point for more detailed comparisons of the ionic equilibrium potentials towards which the transmitter candidates and the intrinsic pathways would drive the membrane properties of the neuron; these data would satisfy the criterion of 'identity of action' (see Werman 1972). These data were also inferential evidence that the transmitter candidate was released by the presynaptic element. Frequently, however, because of the demanding nature of the analysis of equilibrium potentials (see Werman 1972), the 'identity of action' criterion was satisfied by 'pharmacological consistency': synthetic agonists or antagonists, when available, were required to simulate or block, respectively, the responses of the target neuron to both the putative transmitter candidate and to the actual process of transmission. Of these four logical criteria (presence of transmitter, release, identity of action, and pharmacological consistency), cytochemistry or chemical neuroanatomy is directly required to confirm precisely the location of the transmitter substance.

1.2. MONOAMINES

Monoamines were among the first chemically defined transmitters to meet the more rigorous tests as central transmitters. This was due to two specific advantages: the broader armamentarium of drugs available to manipulate the central monoaminergic systems, and the detailed structural information on these systems, thanks to the early development of chemically specific and sensitive methods for their cytochemical localizations (see Moore and Bloom 1978, 1979; and *Volume 1*). However, their unique cellular morphology – with a highly divergent axonal arborization connecting pontine nuclei with cortical regions by routes never visualized by the empirical methods of the metal-impregnation era – and their unique electrophysiological actions – altering membrane potential without increased ionic conductance (see Foote et al. 1983; Siggins and Bloom 1981; Siggins and Gruol 1984, for recent reviews) – required considerable conceptual expansion of ideas of a neurotransmitter (see Bloom 1974, 1975, 1978, 1979, 1984, Madison and Nicoll 1982; Siggins and Bloom 1981; Siggins and Gruol 1984). These nonconventional features, with atypical actions may be outside the boundaries of conditions acceptable to some minds as 'neurotransmitters'. However, due to the unique oxidation chemistry of noradrenaline, it has remained almost the only transmitter directly detectable at the ultrastructural level in its endogenous form and content (see Moore and Bloom 1979).

1.3. PEPTIDES

The most rapidly growing class of neurotransmitter candidate substances, the neuropeptides (Barker et al. 1980; Bloch et al. 1983; Bloom 1983, 1984; Elde and Hökfelt 1979, Fuxe et al. 1979; Hökfelt et al. 1980; Iversen 1983; Morrison and Magistretti 1983; Nicoll et al. 1980a; Rehfeld et al. 1979; Schally 1978; Snyder 1980; Zimmerman 1979), pose an important challenge for this emerging logic of recognized synaptic operations: do

these substances also act in ways generally analogous to amino acids and monoamines or do they represent one or more additional classes of chemical operations by which nerve cells communicate? Although it is now clear that specific peptides fulfill all the logical criteria to be identified as 'the' factors by which specific neurons of the hypothalamus regulate the secretion of specific adenohypophysial hormones (Guillemin 1978; Schally 1978) most of the other known peptides of the central or peripheral nervous system lack rigorous identification as transmitters in general or as messengers for specific central synaptic connections. The strongest case has been advanced for substance P for certain dorsal horn sensory afferents (see Nicoll et al. 1980a) and for luteinizing hormone releasing hormone (Jan et al. 1980) and possibly an enkephalin (Konishi et al. 1981; Wouters and Van der Brecken 1979) in autonomic ganglia. In general, the other peptides are accepted as likely interneuronal messengers because of: (1) immunocytochemical evidence which associates them with specific pathways (often then containing more than one potential transmitter substance) (Chan-Palay 1979, 1981; Hökfelt et al. 1980, 1983); (2) more general neurochemical evidence showing that these peptides are released from neuronal sources by voltage-dependent and Ca-dependent processes (see Iversen 1983 for review, and Buijs and Van Heerikhuize 1982; Bakhit et al. 1983); and (3) occasionally by evidence from ligand binding displacement assays suggesting the existence of functional receptors (see Simon and Hiller 1978; Snyder and Bennett 1976; Snyder and Childers 1979).

An intriguing aspect of neuropeptides is the issue of multiple agonists with similar structures. The grouping of similar sequences of distinct peptide agonists into molecular families (see Blundell and Humbel 1980; Bloom 1983, 1984; Dockray 1979) provides an insight into the possible evolutionary development of specific messenger molecules and their receptors as the nervous and endocrine systems increased in their cellular populations and regulatory complexity. The similarity of sequences also provides a potentially misleading complexity in an era when immunocytochemistry becomes a tool of increasing importance, since antisera raised against one fragment of a given peptide family member may, as a result of structural homology, detect many members. At this point in the emergence of molecular analyses, at least three types of family relationships may be distinguished (see Dockray 1979; Blundell and Humbel 1980).

1. Those in which a common precursor can give rise to multiple different agonists with little similarity in their structures (such as pro-opiomelanocortin (see Bloom 1983), 'big' somatostatin (see Bakhit et al. 1983) or the brain calcitonin-gene related peptide (see Amara et al. 1982; Rosenfeld et al. 1983).
2. Those in which a strong structural similarity relates long domains of peptides, but which rarely occur in the same organisms (such as the substance P family (see Nicoll et al. 1980a)).
3. Those with short domains of structural similarity in which the pro-hormone may contain several copies of identical or highly similar agonists (such as the pro-enkephalins or the prodynorphins (see Bloom 1983), and possibly the pro-VIP (Itoh et al. 1983)).

It seems clear that in the future this molecular inter-relatedness will become of increasing importance in the unraveling of the complex control of neuronal specificity. The new methods of molecular genetics combined with the analysis of the nervous system will therefore become of equally increasing importance (Milner and Sutcliffe 1983).

1.4. IMPLICATIONS

Because the list of putative neurotransmitter chemicals is no longer short, and the range of actions is broad, the tacit assumption that transmitter messenger molecules were functionally equivalent simple excitors or inhibitors is no longer tenable. Had that assumption been validated, the transmitter for a given circuit could have been viewed as irrelevant for intercellular operations aside from the qualitative sign of the circuit and the structural correlates of transmitter-specific circuits might have been undetectable. Since this case does not hold, additional questions can now be addressed to develop further the aspects which might specifically relate transmitter chemistry to cell function and structure.

2. DOMAINS OF KNOWN NEUROTRANSMITTER DIVERSITY

One nihilistic interpretation of neurotransmitter diversity is that the chemical nature for a given neuron is a random selection of the differentiation process and that the specific neurotransmitter (i.e., amino acid, amine or peptide) has no further relevance to the structural or functional properties eventually demonstrated by the neurons which secrete it. While it is clear from the pioneering work of Furshpan, Potter, Patterson (see Patterson 1982) and their colleagues that neurons differentiating in vitro can be diverted from one transmitter designation to another, no such equivalent data have yet been obtained for central neurons. However, it is known that peptide transmitters are often found in CNS regions of embryonic or neonatal brains, where they are undetectable in later post-natal periods (Shoemaker et al. 1983; Bayon et al. 1979; Boer et al. 1980; Emson et al. 1979; Ichihara et al. 1983; Swaab and Boer 1983). Whether this transient expression represents a dying back of misconnected cells (see Cowan 1979) or an epigenetic maturation and switch in the transmitter ultimately employed remains to be determined.

2.1. TIME AND SPACE

What other discriminative features of a chemically labeled neuronal system may be more relevant for abstractions of their function? As I have written elsewhere (Bloom 1973b, 1978, 1979, 1981b), the operations of all neurons can be charted on two domains, space and time, for comparative analysis. The spatial domain of a neuron is the total target cell area to which that neuron sends information. Similarly, the temporal domain is the time course of the neuron's effects on its targets. Let us now ask whether the spatial and temporal domains of chemically characterized neuronal circuits provide any hints to the nature of the operations such circuits may perform.

2.2. STRUCTURAL CATEGORIES

From my perspective as a cellular physiologist interested in broad classes of structural features which may serve to differentiate principles of neurotransmission, I find that most circuits can be lumped into three general categories: (1) hierarchically arranged neurons in chained systematic, or throughput, connections (see Schmitt et al. 1976); (2) divergent, single source, multi-targeted connections (Moore and Bloom 1978, 1979); and (3) local circuit neurons (Rakic 1975).