Neurosecretion– The Final Neuroendocrine Pathway

Edited by
Francis Knowles and Lutz Vollrath

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Preface

This volume marks the end of twenty years of neurosecretion during which there were five symposia, namely Naples (1953), Lund (1957), Bristol (1961), Strasbourg (1966), and Kiel (1970). In comparison with these symposia an exceptionally large number of papers were read at this the sixth symposium, in London, and for economic reasons it has not been possible to publish all the papers *in extenso*. The editors have therefore been obliged to undertake the unenviable work of selection, a task made all the more difficult by the excellence and importance of contributions of the symposium. We felt that it was of the utmost importance at this moment in the history of neurosecretion to present as complete a picture as possible of the present state of the subject in relation to the past and opportunities for the future. We have therefore given some preference to papers with a strong review element, research papers in areas of current importance and contributions which deal with recently developed techniques with promise for the future. We have moreover attempted to strike some balance between the different areas of research on neurosecretion so that the volume as a whole may be of interest to the general reader, and that he will find in it a reasonably coherent pattern of thought which demonstrates neurosecretion as the final neuroendocrine pathway.

We have attempted a certain degree of uniformity of spelling, symbols, etc. but in cases where there is some difference of opinion, as in spelling which differs on both sides of the Atlantic or in a word such as "neuron/neurone" which seems to have no geographical boundaries we have not attempted any value judgements but have left the spelling as it was intended by the authors.

Finally we should like to acknowledge our gratitude to the authors who met our demands in the preparation of manuscripts and especially to those who responded so splendidly to the request that they should not write in their mother tongue but, instead, in English so that the volume could have complete uniformity of language. A special mention should also be made of those who presented results of great importance in the symposium yet are represented in this volume by abstracts only. They knew, and we knew, that their work should be presented in a longer form yet they accepted without complaint the need for selection so that the many areas of research in neurosecretion could each be adequately represented.

Acknowledgements: The members of the VI International Symposium on Neurosecretion are all indebted to the following for financial support of our symposium: Ciba-Geigy (U. K.) Ltd; Hoechst Pharmaceuticals; Imperial Chemical Industries Ltd; Organon International B. V. Some members of the symposium were given financial aid by British Council.

The success of the symposium was in no small measure due to the enthusiasm and efforts of Miss Wendy Charnell and Miss Jeannie Paterson, who did the essential secretarial and art work. The organisers of the symposium are deeply indebted to these and the many other helpers for their assistance.

London, Summer 1974

Francis Knowles
Lutz Vollrath

It is with deep regret that owing to his sudden and unexpected death on July 13th, 1974, Sir Francis Knowles is unable to see the completion and publication of this present volume. Especially so because he found great pleasure and satisfaction in both the organising of the VI International Symposium on Neurosecretion, into which he put so much effort, and in editing the present Proceedings of this conference. Sir Francis' review, published as the Introduction to the present volume, of twenty years of neurosecretion in which he played such an important role reflects very clearly that neurosecretion has lost both a great pioneer and an aristocratic ambassador. He was one of the few who attended all six Symposia on Neurosecretion held so far and he will be greatly missed by all.

Lutz Vollrath

Dr. Manfred Gabe 1916—1973

The sad news of the death of Dr. Manfred Gabe came during the Symposium and his friends thought that it would be appropriate that this volume should be dedicated to his memory, in recognition of his place among the pioneers of neurosecretion.

Dr. M. Gabe, of Rumanian origin, was born in Vienna, Austria on the 28th January 1916. After early studies in Rumania he came to Paris and there studied simultaneously Natural Sciences at the Sorbonne and at the Institute Pasteur and the Faculty of Medicine. After he qualified he became Médecin-externe at The Hôpital de la Pitié until, in November 1942, he was arrested and in February 1943 he was deported to the concentration camp of Golleschau, an annexe of Auschwitz. There for 32 months he cared for the prisoners working in the quarries.

After the war he returned to France and resumed his scientific studies as a director of research at the C.R.N.S.

Dr. Gabe was a man of prodigious energy and skill whose 356 publications included many substantial works, such as the following: –

Histochimie des polysaccharides chez les Invertébrés. Gustav Fischer Verlag, Stuttgart. *Hdb. der Histochemie*, 1962, *2* (1), 95–393, 133 fig.

Neurosecretion. Pergamon, Oxford, 1966, pp. 872, 586 fig.

Neurosécrétion. Gauthier-Villars, Paris, 1967, pp. 1091, 586 fig.

Techniques histologiques. Masson, Paris, 1968, pp. 1113.

Polysaccharides in lower Vertebrates. Hdb. der Histochemie, Gustav Fischer, Stuttgart, 1971, *2* (3), 1–543, 203 fig.

In the field of neurosecretion he will probably be best remembered for his detailed studies on the sinus gland and other invertebrate neurohaemal organs, his histochemical studies on vertebrate neurosecretion and for his discovery of the moulting-gland of crustaceans (named by him the Y organ), as also for his massive reviews of neurosecretion illustrated by his superb histological preparations. In his book Neurosecretion (1966) he wrote, "The charitable silence which one may be tempted to observe when writing an original work would be entirely misplaced in critical surveys, when the aim is to arrive at a true picture". This remark was characteristic of the man, a dedicated pursuer of truth and perfection, who could be merciless in scientific combat yet exquisitely polite and helpful in personal contacts with other research workers. Currently eighty of Dr. Gabe's colleagues and friends are preparing a memorial volume to be entitled "Recherches biologiques contemporaines" to be published in the near future.

In reply to a letter begging him to attend our symposium he wrote, "I have a horror of voyages – I have travelled too much in my youth – but the principal reason for my refusal is that I feel too old and much too tired". We must all feel a sadness that he was not able to make one more voyage to attend our meetings so that we could all have met hime once more and he could have seen the development of the concept he did so much to pioneer. His death was untimely and he will be sadly missed.

Sir Francis Knowles

Contents

IV. Summaries

I. Introduction

Twenty Years of Neurosecretion

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"We have just heard some very interesting things, – and also a great deal of nonsense"!

Those were words that were addressed to Ernst Scharrer, in this city, twenty years ago, when he presented the concept of neurosecretion at a meeting at the Ciba Foundation. They epitomised a not uncommon reaction to the revolutionary idea that a part of the nervous system might have an endocrine function.

Today the words neurosecretion and neurohaemal organs are commonplace in our scientific vocabulary and it is quite difficult to communicate to you the atmosphere – the excitement – of that first conference at Naples in 1953 when this distinct new function of the nervous system crystallized before us. Those have been called the heroic days of neurosecretion and I think it is an appropriate phrase for the founders then encountered a wide spectrum of opposition, ranging from polite doubt to disbelief. Some of us have been privileged to attend the birth of the concept, and then watch its gradual progression – an unfolding of the design.

Before we look at the first symposium at Naples it may be well first to glance at some of the events that led to it. This audience will be familiar with the first report by Speidel in 1919 of what seemed to be glandular cells in the spinal cord of fishes and the startling proposal by Ernst Scharrer in 1928 that the hormones of the posterior pituitary might be made in certain hypothalamic nuclei, a theme pursued by Ernst Scharrer in the 30's in the face of strong opposition. Less generally known is the importance of studies on the invertebrates in those early days. As early as 1917 Kopêc had demonstrated that in Lepidoptera the brain was the source of a secretion necessary to induce pupation. During the 30's and 40's the precise and painstaking experiments of Wigglesworth, Williams, the Thomsens and others combined with the morphological studies by Hanström laid firm foundations for the concept of neurosecretion among the invertebrates.

In the early 30's Frank Brown suggested that in crustaceans the nerve cord might be, in addition to the eyestalk, a source of hormones controlling the chromatophores and my good friend Kleinholz suggested to me a critical experiment to disprove this heretical idea. I carried out this experiment – but obtained evidence strongly supporting Brown. That was my first publication and I must say that I have been a convert to neurosecretion ever since!

I have spoken of early workers on invertebrate neurosecretion but I have reserved at the end a very special place for Berta Scharrer and the great contribu-

tions she made to insect physiology and the neurosecretion concept. It must be very rare, if not unique in the history of science, to find such a husband and wife team with interests so perfectly complementary for the formulation of a new concept. Ernst on the vertebrates – Berta on the invertebrates – how excited they must have been as they found new correlations in their studies and saw correlations in the work of others substantiating the universality of their neurosecretion concept. The late 1940's were significant for neurosecretion for then the Scharrers published an important review, Harris and Green enunciated their releasing factor theory and in 1947 a conference on arthropod endocrinology was held in Paris, at which many papers dealt with neurosecretion.

From Hanström and Cazal we heard the confirmation and extension of the discovery by Berta Scharrer a few years before that nerves from the pars intercerebralis of the insect brain contained secretory material. From Carrol Williams we heard that experiments had shown that the pars intercerebralis cells of the insect brain produce a substance which triggers the release of a prothoracic gland hormone essential for development. Clearly neurosecretion could be a vital link between the nervous and the endocrine systems. It was not long before Berta Scharrer showed by lesion experiments in insects that neurosecretory material passed from the brain along the pericardial nerves, thus demonstrating in an invertebrate a passage of neurosecretory material along a nerve, as had been postulated by Ernst Scharrer but not yet demonstrated in vertebrates.

The situation was soon to be transformed by the publication in 1949 of Bargmann's inspired discovery that the Gomori Chrome-alum-haematoxylin stain clearly differentiated the hypothalamic neurosecretory system in the mammalian brain and made possible the detection of neurosecretory material along the course of axons. Soon this method was to be used by many investigators and particularly in Bargmann's school where the names of Hild, Eichner, Kratzsch, Ortmann, Rodeck, Zetler, Schiebler, Knoop and Thiel will be remembered for their contributions. Soon the evidence for vertebrate neurosecretion became overwhelming and in the spring of 1951 Bargmann suggested to Ernst and Berta Scharrer that the time had come to bring together investigators in the field and lay firm foundations for the concept of neurosecretion. Accordingly we met in Naples.

Naples 1953. At the Naples conference the concept of neurosecretion in vertebrates was dominated by the "classical" neurosecretory pathway, namely the hypothalamo-neurohypophysial system. Yet coming events are said to cast their shadows before them and it is interesting to see how some further developments in neurosecretion were adumbrated at that first symposium. Benoit and Assenmacher for example described fine droplets of gomori staining material in the external zone of the median eminence of birds. Hanström drew attention to a probable neurosecretory activity of the nuclei tuberis, the nerve fibres of which constitute the external layer of the median eminence and do not form part of the classical neurosecretory pathway.

There was therefore already then some morphological support for the Harris-Green postulate. On the other hand the experimental evidence for neurosecretory control of the pituitary was confusing. From Birmingham Sir Solly Zuckerman brought news of ferrets which succeeded in maintaining normal sexual cycles

though it was claimed that the portal vessels from the hypothalamus to the pituitary had been effectively interrupted but Benoit and Assenmacher reported contradictory results in comparable experiments on ducks, and there was therefore at that time an aura of uncertainty over the status of neurosecretion in pituitary pars distalis control.

The evidence for a neurosecretory control of endocrine function in insects was far more convincing. The results which had been reported in Paris some years previously were greatly extended by the Thomsens, De Lerma, Bounhiol, Arvy,

Fig. 1. Naples, 1953; at the Stazione Zoologica [Left to Right: E. Scharrer, W. Bargmann, B. Scharrer, R. Dohrn (Director of the Stazione Zoologica), J. Benoit]

Gabe, Possompès, Grandori and others, all of whom demonstrated clearly the morphology of that neurosecretory system which passes from the pars intercerebralis to the corpora cardiaca-allata complex. Neurosecretory systems in crustaceans also were described in detail by Enami, Passano and others.

It is noteworthy that approximately 60% of the papers at that first neurosecretion symposium dealt with invertebrates. Also that the emphasis was on the morphological characteristics of the neurosecretory cell. One carried away a conviction that early in evolution a special kind of nerve cell had appeared which showed features characteristic of gland cells such as the elaboration and discharge of colloid droplets in addition to the general features of neurones. These special cells were organized into distinct groups and furthermore the apparent universality of the Gomori stain to pick out neurosecretory systems in vertebrates and invertebrates further supported the view that neurosecretion could be recognised as a clearly definable sub-function of the nervous system.

At the same time Ernst Scharrer emphasised that neither the Gomori nor the equally effective aldehyde fuchsin stains were cytochemical in value and pointed

out that the terms Gomori substance, Gomori positive and Gomori negative, were not to be recommended. These very wise remarks were unfortunately not heeded by later workers, many of whom used these staining methods alone as criteria for neurosecretory activity.

To some of us at Naples it seemed that not only the morphological features of neurosecretory cells were significant criteria for identification but also that the relation of neurosecretory neurons to the body fluids might distinguish them from other elements of the nervous system. Stutinsky drew attention to the fact that in the eel neurosecretory perikarya projected into the c.s.f. of the third ventricle. A few weeks before the symposium Carlisle and I had proposed the name neurohaemal organ to describe the collections of endings of neurosecretory fibres abutting on blood-vessels in crustaceans, and at Naples I suggested that a definition of neurosecretion might include the neurohaemal concept.

Lund 1957. Four years later we met at Lund, as guests of our much-loved Bertil Hanström. From the start the neurohaemal concept became a matter of controversy. The intervening years had shown that the stainability of neurones by itself was not a reliable guide to their endocrine properties. Perhaps for this reason Ernst Scharrer was anxious that we should officially define neurosecretion (at the Lund Symposium) and that the discharge of hormones at neurohaemal organs would be a fundamental feature distinguishing neurosecretory neurones from what our French colleagues charmingly call "neurones banales". Wigglesworth opposed this for he felt that to make a formal definition at that stage would be premature.

Eventually no formal definition was passed by the Symposium but we all agreed in our own minds that at least a working definition of neurosecretion was clear. Cytologically neurosecretory neurones combined features of neurones and gland cells and their axon terminals did not appear to make synaptic junctions with effector organs but instead discharged secretory products into the circulation at a neurohaemal organ.

The Lund Symposium confirmed and extended many of the observations at Naples. By now the electron microscope had revealed small vesicles, some 1000 Å – 3000 Å in diameter in neurosecretory perikarya and axons, as was demonstrated by Bargmann.

A new precision was introduced by Sloper who demonstrated a histochemical test for neurosecretory material and a radioisotope method for studying the dynamics of the process, both dependent on the presence of cysteine. He was able to show that neurosecretory material moves down the axon in fine particulate form.

The discovery of elementary neurosecretory vesicles presented opportunities but also problems. Did these vesicles contain the hormones or had they some other function? Where were they produced? Were these vesicles perhaps a feature which would distinguish neurosecretory neurones from other elements of the nervous system? These were themes which dominated the next symposium at Bristol in 1961.

Bristol 1961. The problem of the identification of vesicles with hormones was very elegantly resolved by Heller and Lederis who combined methods of centrifugation, bioassay and electron microscopy to show that the oxytocin and vasopres-