Morphological Aspects of Neurons as Secretory Cells

Yutaka Sano

Department of Anatomy, Kyoto Prefectural University of Medicine, Kyoto, Japan

**Summary.** The concept of a neurosecretory system consisting of a secretory center (production of bioactive substances in neuronal soma), a transport pathway (transport by axonal flow) and a neurohemal organ (storage and release at nerve endings) has been established through a series of histological studies on the hypothalamic magnocellular nuclei by two pioneers, SCHARRER and BARGMANN, together with their co-workers.

In the early stages of the investigation, these actively secreting neurons were considered as exceptional neurons, separated from ordinary ones. However, since the application of modern techniques, such as electron microscopy, immunohistochemistry, and in situ hybridization, have made progress in tracing the cytological course from the gene expression to the release of bioactive product, it has become obvious that the classical definition of neurosecretion must be extended to include a variety of neuroepithelial derivatives, such as amineergic and peptidergic neurons and also paraneurons. At present, all neurotransmitters and neuromodulators can be regarded as secretory substances produced in neurons.

Newly developed precise techniques, e.g., a tracing method with a computer graphic system for the demonstration of axonal arborizations, have led to a dramatic change in our understanding of the fine details of cytological features, offering much more complicated structures than images presented by the classical impregnation technique. Immunohistochemically demonstrated serotonergic neurons in the brain have revealed enormous reticular extensions and anastomoses of beaded processes which were previously unknown. Neurons composing the nervous system show greatly varied shapes and structures. This morphological heterogeneity makes possible the diversity of the brain function.

This review stresses that advances in studies in the field will be promoted by a dual strategy: one to investigate neurons in their general features especially the secretory aspects, and the other to pay attention to the special features of each variety of neurons.

**NEUROSECRETION STUDIES**

While Ernst SCHARRER was studying photosensitivity in *Phoxinus laevis* L., the minnow, with enucleated eyeballs, he noticed that nerve cells composing the preoptic nucleus of the diencephalon contained in their perikarya vacuoles and stainable granules of various sizes. SCHARRER (1928) assumed that these cells would have a secretory activity similar to that of gland cells. This is regarded as the first recognition of the phenomenon, neurosecretion.

Immediately after World War II, BARGMANN (1949) succeeded in the selective staining of secretory products in neurons of the magnocellular nuclei, i.e., the supraoptic and paraventricular nuclei of the hypothalamus, by applying the chrome hematoxylin phloxine staining which was originally developed by GOMORI (1941) to demonstrate β cells of the pancreatic islets. BARGMANN and his co-workers observed the effects of water balance changes and sectioning of the hypophysial stalk about a stainable material, and disclosed that these neurons produce posterior lobe hormones, i.e., vasopressin and oxytocin, in the perikarya, transport them transaxonally and release them into the blood vessels in the posterior lobe of the pituitary gland (HILD, 1951; ORTMANN, 1951; HILD and ZETLER, 1953).

Structures analogous to the hypothalamohypophysial neurosecretory system were later found in fish spinal cord, insect brain and crustacean optic stalk (SCHARRER, B., 1952; ENAMI, 1959; WELSH, 1959; SANO, 1961). These are known, respectively, as the caudal neurosecretory system, inter-cerebralis-corpus cardiaicum-allatum system and X-organ-sinus gland system. In invertebrates the hormones produced in these systems are involved in molting and pupation. Through such studies, neurosecretion has come to be recognized as one of the essential regulatory mechanisms of the body.

Since the latter half of the 1950s, electron microscopes have been applied to biomedical research.
Submicroscopic studies first revealed that the cytoplasm of the neurosecretory neurons contains many membrane-bound, highly electron dense granules approximately 200 nm in diameter (FUJITA, 1957; SANO and KNOOP, 1959; PALAY, 1960). Subsequent studies have shown that these granules involve peptides with hormonal activities, that the peptides are synthesized in rough endoplasmic reticulum to be packaged with unit-membrane in the Golgi apparatus, and that the peptides are transported by the axonal flow to be released by exocytosis at the axon terminal.

Electron microscopes have not only made it possible to explore ultrastructures but have greatly changed the purpose of morphological studies. Previously, morphologists attempted to clarify structural and functional differences among cells and tissues. The focus now is on determining which fundamental and common structures exert a particular function. Electron microscopy elucidated that kinetic changes in structure, ranging from production to release secretory products in neurosecretory cells, are in fundamental agreement with those in protein-secreting gland cells. The only difference between these two types of cells lies in whether or not a long axon is inserted in the space between the cell body, i.e., the site where secretory materials are produced, and the nerve ending, i. e., the site where they are released.

**STUDIES ON BIOACTIVE AMINES**

In 1962 FALCK et al. were successful in devising a formaldehyde-induced fluorescence histochemistry for the microscopical detection of biogenic amines. This method has rapidly contributed to clarifying the histological composition of monoaminergic neurons which are distributed in the central nervous system. Prior to the study of aminergic neurons, neurosecretion was regarded as a phenomenon characterizing a few exceptional neurons. Studies on chemical transmission, which began early in this century with the works of ELLIOTT, (1904), DALE (1914) and LOEWI (1921), have also been relatively indifferent to the study of neurohormones. However, with the advances in the investigation of amine neurons in the central nervous system, it became increasingly clear that the mechanism involving the synthesis, transport and release of amines in such neurons is closely similar to that in neurosecretory cells, leading to a revision of existing concepts regarding nerve cells.

Amines, particularly noradrenaline, are neurotransmitters that have been investigated for a long time. They are released by typical nerve cells, not by exceptional, specific nerve cells like neurosecretory cells. For this reason, hormonal production in neuroendocrine cells and neurotransmitter production in conventional neurons have been discussed in conjunction with each other since the latter half of 1960s.

**STUDIES OF BIOACTIVE PEPTIDES**

In 1944, HARRIS’ pioneering thesis work for Cambridge University indicated that hypophysiotrophic messenger substances produced in the hypothalamus are transported through the hypophysial portal veins to the anterior lobe, where they exert neural control on secretory activity (HARRIS, 1948). Subsequently, two research groups led by SCHALLY and GUilleMIN worked for a quarter of this century to extract and determine the chemical structure of the thyrotropin-releasing hormone, confirming HARRIS’S hypothesis...
In the 1970s stimulatory and inhibitory neuropeptides controlling the function of the adenohypophysis, i.e., regulatory hormones, such as luteinizing-hormone releasing hormone, somatostatin, and corticotropin-releasing hormone, were successively isolated and characterized.

Immunohistochemistry, which was established by Coons et al. in 1942, was popularized by Sternberger’s development of the peroxidase-antiperoxidase method (1974), and soon enabled morphologists to identify the in vivo distribution of newly discovered active peptides. The localization of parvocellular neurons producing the above regulatory hormones in the hypothalamus was achieved in various animals in the 1970s, and these parvocellular neurons have become recognized as a sort of neurosecretory cell.

New active peptides have been extracted on a regular basis and isolated from various organs such as the digestive tract, skin, myocardium and brain, owing to rapid advances in peptide chemistry. The number of bioactive peptides characterized so far exceeds 100, and the search for “new peptides” continues. It has been disclosed that some neuronal peptides are present in gastrointestinal and other endocrine cells, and in contrast, many peptides obtained from the gut and endocrine organs are present in the central nervous system. These are now called “brain-gut-hormones”. As the cardiac muscle cells have been demonstrated to produce atrial natriuretic peptide, ANP, one many coin the term “brain-heart hormones”. (A new ANP analogous brain natriuretic peptide was isolated by T. Sudo et al. from acid extracts of porcine brains—Nature 332: 78-81, 1988)

Neuropeptides are widely distributed in the animal kingdom, including protozoa. A number of mammalian neuropeptides can be shown in the nervous system of invertebrates. Conversely, some invertebrate bioactive peptides, i.e., FMRF amide, growth-promoting “head activator” of Hydra etc., are present in the mammalian brain. Recently the existence of a sodium-potassium-ATP-ase inhibiting natriuretic factor in the hypothalamus was immunohistochemically detected using the digoxin-antibody. Digoxin is one of cardiac glucosides extracted from the leaf of foxglove. This chemically unknown factor, a so-called endogenous digitalis-like substance, is noteworthy for the study of the evolutionary aspect of neurotransmitters (Yamada et al., 1987; Ihara et al., 1988).
SECRETORY MECHANISMS AND MOLECULAR BIOLOGY

Secretion was once thought to be a function specific to epithelial cells, but in recent years this concept has been interpreted more broadly; even mesenchymal cells that release synthesized substances extracellularly, e.g., fibroblasts and osteoblasts producing collagen etc. are seen as similar to gland cells. In this context, neurons that synthesize transmitters and release them to the synaptic cleft, fully deserve to be included in the category of gland cells.

The tremendous progress in molecular biology over the last decade has enabled us to demonstrate the precise localization and identification of individual cells that contain a specific nucleic acid sequence. This method, in situ hybridization, is a powerful technique for detecting DNA or RNA in a manner analogous to the immunohistochemical localization of cells containing a particular protein or peptide (PARDUE and GALL, 1969; JOHN et al., 1969). Application of this in situ hybridization technique permits discrimination between a site of peptide biosynthesis and its storage or uptake. In other words, this method can be applied to ward the study of a specific gene expression following physiological or pharmacological manipulations by using a specific probe. For example, the selective changes in oxytocin messenger RNA levels in the paraventricular nucleus occur after estrogen and progesterone treatment. Silver grains over the cytoplasm reflect the presence of oxytocin messenger RNA; the number of grains changes following sex steroid treatment, suggesting that an oxytocin gene in hypothalamic neurons is expressed by such a stimulus (KAWATA et al., 1988a, b).

MORPHOLOGICAL REEVALUATION OF NEURONS

Neurological study has its own long history. As is generally recognized, it started as a field of modern science with the discovery of the silver impregnation method by Camillo GOLGI (1873). The impregnation method enables us to clearly visualize perikarya and the two kinds of processes, dendrites and axons. Most neurobiologists believe that the image of black impregnated neurons truly represent all aspects of neurons existing in situ. Simplifying the images, a
model scheme of a neuron can be produced, but recent studies have revealed that no such neuron with the simple processes as seen in the model exists.

Recently methods by which neurons are labeled by the intracellular iontophoretic injection of tracer substances using a micropipette electrode have become remarkably developed. The three-dimensional reconstruction of axonal arborizations of the labeled neuron can be produced by tracing it with a computer graphic system. The image of a neuron yielded by these modern methods is more complicated than that obtained by the impregnation method, and is highly variable. These results suggest a possibility that the neuronal image obtained so far by the silver impregnation have not reflected the in situ organization, indicating a great disparity between the image of the impregnated neuron and that of the real neuron.

The immunohistochemical study of amine neurons revealed them to have long processes with many branchings, taking neuroanatomists by surprise. Robert Moore (1982) expressed the morphological structure of noradrenergic neurons as a "highly-collateralized axon system with extensive terminal plexuses." The distribution of serotonin fibers in the brain is much denser and more extensive than that of noradrenaline fibers. The wide-spread three-dimensional networks composed of serotonin fibers are observed in almost all areas of the central nervous system except for thick myelinated pathways, such as the optic tract or the pyramidal tract (Sano et al., 1982; Takeuchi, 1988).

In the terminal regions serotonin fibers surround the cell body and dendrites of each neuron. This basket-like pericellular network extends from one neuron to another along the course of thick dendrites in an Ivy-like fashion, and many neurons, located sporadically in the nuclear area, are entwined with a network of serotonin fibers (Sano and Ueda, 1988).

Serotonin fibers are distributed not only in the parenchym of the central nervous system, but also along the wall of blood vessels in the subarachnoid space and in the ventricular lumen as a supraependymal plexus (Matsuura et al., 1985). Serotonin fibers branch off and anastomose at the points corresponding with varicosities, and from a true meshwork. This reticulum is apparently different from the so-called "telodendron" which has been visualized as the terminal portion of conventional neurons.

Unsicker et al. (1974) first recorded a study on the differentiation of adrenomedullary cells in vitro. They observed that when the adrenal chromaffin cells are cultured in a nerve growth factor (NGF) containing medium, most cells grow processes and differentiate into neuron-like cells substantially similar to sympathetic neurons. Recently we also carried out an immunohistochemical study of the adrenomedullary cells from neonatal rats in vitro for the purpose of examining the structure of the outgrowing processes. In this work we found that chromaffin cells differentiate into two different types of neuron-like cells: type A cells possessing simple straight processes, and type B cells having complicated winding processes. Immunohistochemically the former corresponds to tyrosine-hydroxylase-reactive cells and the latter, serotonin-positive cells. These results suggest that there is an essential difference between the axonal ramification pattern of catecholaminergic neurons and that of serotonergic neurons.

Both endocrine and nerve cells may share the mechanisms of transmitting information to target cells with substances produced and released by the cells. However, the morphological variety of neurons indicates a variety of transmission patterns, making the study of generalized functions difficult.

Today, we have extensive data on various neuronal mechanisms, e.g., the conduction of impulses, neurotransmission, axonal flow, plasticity, etc. Until the specific features of each neuron type among the greatly varied forms are determined and until our images on neurons formed so far by simplified model schemes are removed, we will not be able to approach a real understanding of the marvelous organ, the brain and of the elaborate regulatory mechanisms of the body.

REFERENCES


Prof. Yutaka Sano
Department of Anatomy
Kyoto Prefectural University of Medicine
Kawaramachi-Hirokoji, Sakyo-ku
Kyoto, 602 Japan