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Structure-Function Correlation in Mammalian Neurosecretion

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I. Introduction

There can be no doubt that neurosecretory systems are important in mammalian physiologic mechanisms. Two principal neurosecretory systems occur in the mammalian hypothalamus. The "magnocellular" neurosecretory system projects from the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus to the neural lobe and produces oxytocin and vasopressin. The "parvicellular" system projects to the median eminence and secretes releasing and inhibiting hormones that influence the output of all adenohypophyseal hormones. In this way, it modulates the activity of the majority of peripheral endocrine organs. Both systems are located primarily in the hypothalamus, which is thus a major neuroendocrine regulatory

center. The magnocellular system has been defined anatomically and accepted as a neurosecretory system for more than 20 years, but the first light-microscopic identification of the perikarya of specific neurons of the parvicellular system was as recent as 1973 (Barry *et al.*, 1973). Inevitably, therefore, most work on neurosecretion has centered on the magnocellular hypothalamoneurohypophysial system (HNS).

There have been many reviews of the enormous literature on neurosecretion in the HNS. Our purpose in this article is to review that part of the literature that enables us to correlate changes in ultrastructure of the HNS with changes in its functional activity.

Morphological investigations have played a large part in the acceptance of neurosecretion as a physiologic process. The activities of the two secretory products of the HNS—vasopressin (ADH) and oxytocin—were first demonstrated at the turn of the century. Oliver and Schäfer (1895) first described the vasopressor properties of pituitary extracts, and pressor activity was localized to the posterior pituitary by Howell (1898). The antidiuretic properties of vasopressin were initially obscured by the diuresis that resulted from the pressor effect, but Farini (1913) and von den Velden (1913) were both struck by the association of diabetes insipidus with lesions destroying the pituitary. Both authors showed that the urine flow of patients with diabetes insipidus could be reduced by injection of posterior pituitary extracts. The oxytocic activity of such extracts was first reported by Dale (1906, 1909), and the milk ejection activity by Ott and Scott (1911) and Gaines (1915).

During this period, Cajal (1894) established that the posterior lobe had a profuse innervation from cells in the region of the optic chiasm in addition to its cellular component, the pituicytes (Berkley, 1894; Bucy, 1930). In 1908, Herring described small hyaline bodies in the posterior pituitary and correctly suggested that these were accumulations of hormone-containing material. However, at this time it was thought that the secretion was produced by cells of the pars intermedia (Cushing and Goetsch, 1911).

Neurons with morphological characteristics of secretory cells were first described in the spinal cord of the skate by Speidel (1919), but it was in the period 1930–1950 that E. and B. Scharer pioneered the concept of neurosecretion against considerable opposition (see reviews in Scharer and Scharer, 1937, 1940, 1945, 1954). During this period Gersch (1937, 1939) proposed that the secretory products of the posterior pituitary were formed as lipid droplets by the pituicytes. The increased numbers of lipid droplets in appropriately stimulated animals were interpreted as increased hormone formation in response to increased demand. Since destruction of certain parts of the hypothalamus, notably the SON (Bailey and Bremer, 1921; see Fisher *et al.*, 1938), lead to a condition of diabetes insipidus, it was generally believed that the pituicytes were under the control of secretomotor nerves from the hypothalamus (Ranson and Magoun, 1939; Gersch and

Brooks, 1941). However, the pituicytes were not universally accepted as the sole site of synthesis because removal of the neural lobe did not lead to a permanent diabetes insipidus (Gagel and Mahoney, 1942; Keller, 1942). Furthermore, there were more axons in the pituitary stalk than would have been necessary to innervate the pituicytes (Rasmussen, 1938), and many of the nerve fibers ended not on pituicytes, but on blood vessels (Vazquez-Lopez, 1942).

It was essentially a technical breakthrough that led to the acceptance of the Scharrer's concept of neurosecretion. In 1949 Bargmann (1949a,b) applied Gomori's (1941) chromalum-hematoxylin (CAH) stain to the HNS and was able to demonstrate stainable "neurosecretory material" (NSM) throughout the system. This work, and subsequent studies using the technique, demonstrated that NSM was produced in the perikarya of the magnocellular neurons and transported down the axons of these cells through the pituitary stalk to the posterior pituitary, from which it could be released by appropriate stimuli (Bargmann and Hild, 1949; Bargmann and Scharrer, 1951; Hild and Zetler, 1951, 1953; Ortmann, 1951, 1960; Stutinsky, 1951; Scharrer and Scharrer, 1954).

It is clear, therefore, that by about 1955 the essential anatomy and function of the HNS was well defined. Since that time it has again been technical advances that have permitted further probing of the unanswered questions. The development and use of the electron microscope has been of major importance, though all too often investigators have relied on a subjective visual assessment rather than objective quantification to determine changes in organelle populations. Since samples are inevitably small, organelles non-uniformly distributed, and the cells and the neural lobe change in size, visual estimation is often inadequate, and proper quantitative assessment should be applied wherever possible.

Radioactive tracers have been used with light or electron microscopy in autoradiographs, as radioactive precursors and labels for biochemical studies, and also to monitor ionic movements during hormone release. *In vitro* techniques have been developed that permit study of the isolated neural lobe and, more recently, the entire HNS. Specific antibodies to the neurohypophysial hormones and their associated "neurophysin" proteins have lead to the development of extremely sensitive assays for the hormone and, more recently, to their use for immunocytochemical localization of hormone and neurophysin. Finally, the development of electrophysiological techniques has helped to elucidate the relationship between electrical activity and hormone release, and it now appears that oxytocin and vasopressin cells are definable electrophysiologically as well as immunocytochemically.

As we shall attempt to show, investigations using these techniques alone and in combinations have shed considerable light on the morphological correlates of the synthesis, storage, transport, and release of vasopressin and

oxytocin. Although there are many similarities between these processes in the oxytocin and vasopressin cells, there are also substantial differences. Thus it would be unreasonable to expect the parvicellular neurons to be physiologically identical with magnocellular neurons. There are certain similarities, such as the packaging within granules of some of the hormones, but there are also marked differences, such as the size of the neurons and the much smaller storage of hormone in the neurovascular contact area.

We hope, however, that a clear understanding of the morphological correlates of neurosecretion in the magnocellular system will, at least, be of use in the design of experiments on the parvicellular system.

II. Light Microscopic Correlates of Neurosecretion

As we have seen, Bargmann's (1949a,b) light microscopic identification of NSM by specific CAH staining was a major factor in the acceptance of neurosecretion as a physiological process and, for almost a decade, most studies of structural aspects of neurosecretion were made using the light microscope. These studies have been reviewed in detail by Sloper (1958) and Ortmann (1960), and we shall therefore review only the main conclusions for later comparison with the ultrastructure.

A. STAINING OF NEUROSECRETORY MATERIAL

Bargmann's (1949a,b) work was soon confirmed, and extended. Smith (1951) compared the results of staining with the CAH technique with those obtained with the older Masson stain. He concluded that the large spherical and irregular CAH-stained masses were the equivalent of the large spherical and irregular fuchsinophilic masses described by Scharrer (see Scharrer and Scharrer, 1945). However, the CAH technique also demonstrated fine granules, $0.2\ \mu\text{m}$ in diameter, of NSM. The paraldehyde fuchsin stain gave similar results to CAH (Gabe, 1953), and both Barnett (1954) and Adams and Sloper (1955, 1956) demonstrated that histochemical methods for detection of sulfhydryl ($-\text{SH}$) groups also stained neurosecretory material intensely. At that time it was thought that the intense staining detected the $-\text{SH}$ groups of the posterior pituitary hormones. However, Hild and Zetler (1953) had shown that stainable NSM, but not hormonal activity, could be extracted by alcohol. It now seems likely that such stains primarily detect the sulfur-rich neuropeptide proteins of NSM.

At about the same time, Palay and Wissig (1953) demonstrated small granules by phase-contrast microscopy of supravital preparations of supraoptic neurosecretory cells. These granules were similar in size, number, and position to the fine granules stained by CAH, and were distinguishable from

the more rod-shaped mitochondria by their rounded shape and by the fact that they did not stain with the mitochondrial stain Janus green (Schiebler, 1951).

Light microscopic staining of NSM, although most important in establishing the concept of neurosecretion, left three main areas of uncertainty. Since neuronal boundaries could not be defined, it was unclear whether NSM was exclusively intracellular, particularly in the neural lobe. The fine CAH-positive granules were at the limit of detectability by the light microscope, and so little further could be deduced about the nature of subcellular storage of NSM. Finally, although attempts were made, it proved difficult to quantify NSM by photometric means.

B. GENERAL APPEARANCE OF NEUROSECRETORY CELLS

From these studies emerged a general picture of neurosecretory cells as large neurons with few processes, eccentric nuclei, prominent nucleoli, and profuse cytoplasm. The Nissl material was usually peripheral and, although it usually appeared amorphous, was striated when viewed by phase contrast in supravital preparations (Palay and Wissig, 1953). Within the perikarya the secretory granules were located primarily in the perinuclear region, while in the varicose axons the NSM was located peripherally around a central NSM-free core. Wide variation in the NSM content of the cells was common. Large irregular vacuoles have been reported in the cytoplasm of neurosecretory cells of humans (see Palay, 1953) and dogs (Verney, 1947; Jewell, 1953). They achieved a brief prominence because Verney (1947) interpreted them as osmoreceptors, but their isolated occurrence in only two species argues against this interpretation.

Transport of NSM from the perikarya of the neurosecretory cells to the neural lobe is an essential part of the concept of neurosecretion. Specific staining techniques had demonstrated NSM throughout the axons and in the neural lobe, but a demonstration of centrifugal movement of NSM was crucial. The phenomenon of axoplasmic flow was known from the work of Weiss (1944). Centrifugal transport of NSM was first demonstrated experimentally by Hild (1951a,b), who showed that, if neurosecretory axons were obstructed, there was a substantial accumulation of NSM on the cell body side of the obstruction.

Many subsequent experiments have confirmed the accumulation of NSM proximal to a transection of the supraopticohypophysial tract (see Ortmann, 1960). The accumulation of NSM that can occur immediately distal to the transection is almost certainly a result of redistribution of NSM rather than *de novo* formation in the neural lobe (see Section VII, A, 2, b).

A light microscopic autoradiographic approach to the transport question was adopted by Sloper (1958; Sloper *et al.*, 1960; Sloper and King, 1963). Intracisternally injected radioactive cysteine could be located first in the