Ultrastructural Study of the Pericardial Organ-Anterior Ramifications Complex Neurosecretory Terminals*

Peter M. Andrews

Department of Cell Biology, The University of Texas Southwestern Medical School at Dallas, Dallas, Texas, U.S.A.

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Summary. The pericardial organ-anterior ramifications complexes of *Uca pugilator* and *Callinectus sapidus* were studied by transmission electron microscopy. Five morphologically distinguishable groups of granules and two groups of vesicles were identified. These granules and vesicles are present in approximately the same proportions in the pericardial organs and anterior ramifications of both species. Two of the granule groups are never mixed in the same axon terminals and are believed to represent different hormone-protein complexes. The remaining granule and vesicle groups are believed to be products of neurosecretory hormone release. Evidence that at least some of these granules and vesicles arise from intraaxonal release of neurosecretory material is presented.

Key words: Pericardial organ — Anterior ramifications — Neurosecretion — Electron microscopy.

Introduction

The pericardial organs (PO) and anterior ramifications (AR) are two important neurohormonal storage organs (neurohemal organs) found in the thoracic cavities of crustaceans. The PO consists of a rather large nerve plexus at the openings of the brachiocardiac veins in the lateral pericardium. The AR is a much smaller nerve plexus which lies beneath a sinus membrane separating the ventral respiratory muscles from the dorsal thoracic cavity. Because the AR arise from one of the three nerve trunks (first segmental nerve) which also gives rise to the PO, the AR and PO are often referred to collectively as the pericardial organ-anterior ramifications complex (POARC) (Maynard, 1961a). Neurosecretory hormones that are synthesized in cell bodies of the thoracic ganglion, the POARC itself (Maynard, 1961b) and the circumesophageal ganglion (Cooke and Goldstone, 1970) are transported within membrane-bounded granules along axons to the POARC for storage and ultimate release into the blood. Thus far, there is no published information as to the functional significance of the AR. Studies of the PO, however, have shown that this organ contains a cardioexcitatory peptide hormone (Smith, 1947; Alexandrowicz and Carlisle, 1953; Cooke, 1964; Maynard and Welsh, 1959; Berlind and Cooke, 1970) associated with neurosecretory-like granules (Terwilliger et al., 1970). It has also been suggested that since the pericardial organs are in a suitable position for release and immediate distribution of material over the entire body, this neurohemal organ might serve some more generalized functions other than cardioregulation (Maynard and Welsh, 1959).

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Thus far there have been no published ultrastructural studies of the AR and only three rather brief electron-microscopic reports on the PO (Knowles, 1962, 1963; Maynard and Maynard, 1962). The investigation described herein was therefore undertaken to study the ultrastructural morphology of POARC neurosecretory terminals in greater depth using the advantages of current electron-microscopic techniques. In that different hormone-protein complexes may be stored in morphologically distinguishable types of neurosecretory granules (Bunt and Ashby, 1967), it was hoped that this study might give clues concerning (1) the possible presence of a variety of neurosecretory hormones in the POARC and (2) whether the PO and AR release the same or different hormones.

Perhaps more important, however, is the fact that the POARC represents a system in which the controversial mechanism of release of neurosecretory hormone may be studied. Many, if not most, previous ultrastructural descriptions of the release of neurosecretory hormones have been based on studies that subject neurohemal organs to electrical shock or to other intense physiological trauma in order to induce massive discharge of hormone (Palay, 1955, 1957; Hartmann, 1958; Gerschenfeld et al., 1960; Fujita and Hartmann, 1961; Fridberg et al., 1966; Monroe and Scott, 1966; Bunt and Ashby, 1968; Norman, 1969; Scharrer and Kater, 1969; Scott, 1969). The results of these investigations have recently been questioned as possibly producing artifacts arising from intense and abnormal inducement of hormone release (Douglas et al., 1971). The gross morphology, location in the body and function of the PO, on the other hand, all suggest that this organ normally releases significant amounts of neurosecretory material into the blood. The PO therefore should exhibit many ultrastructural characteristics of neurosecretory-hormone release without having to subject these organs to abnormal trauma.

Materials and Methods

The animals used during the course of this study were adult male and female intermolt blue crabs, Callinectes sapidus, obtained from Lake Pontchartrain, Louisiana, and adult male and female intermolt fiddler crabs, Uca pugilator, obtained from Panacea, Florida. Both species were studied during the months of April, May and June. The pericardial organ-anterior ramifications complexes (POARCs) of these crabs were fixed in situ by injecting 6% glutaraldehyde buffered with sodium phosphate (Sabatini et al., 1963) directly into the thoracic cavity. The osmolarity of the fixative was adjusted to 1000 milliosmoles by addition of sucrose (Caulfield, 1957). After ten minutes, the POARCs were excised and immersed in the 6% glutaraldehyde fixative for four to six hours. Following rinsing in phosphate buffer, the tissues were postfixixed for one hour in 1% osmium tetroxide. The tissues were then rinsed in 20% acetone and water and stained en bloc in 0.1 M s-collidine buffered 0.5% uranyl acetate at pH 4.5 for 12 hours in the dark. Following treatment with uranyl acetate, the tissues were dehydrated through an ethanol series to propylene oxide and embedded in a mixture of Epon and Araldite resins. Ultrathin sections (50-80 nm), sometimes poststained with uranyl acetate and lead citrate (Reynolds, 1963), were examined in a Philips 200 electron microscope operating at 60 kV.

Estimates of the relative occurrence of various neurosecretory granules and vesicles were based on surveys of the POARCs from 12 male and 12 female blue crabs and from 12 male and 12 female fiddler crabs. Each survey consisted of examining sections from several more or less arbitrary areas along the anterior, posterior and longitudinal bars of the pericardial organs and along the proximal, intermediate and terminal segments of the anterior ramifications.