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# Reassessment of secretion manner from neurosecretory terminals in the rat posterior pituitary

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## Abstract

**Secretion manner from neurosecretory terminals was revalued in the rat posterior pituitary (PP). 1 μm-thick sections stained with toluidine blue showed various structures in the PP, such as fibers, pituicytes, capillaries and vascular spaces. Although a lot of terminals containing small clear vesicles and large dense core vesicles (LDCV) were observed at the electron microscopic level, morphological changes associated with exocytosis were never found around the membrane of terminals. It was of particular interest that terminals occasionally showed protrusions of membrane containing electron dense substance. The protrusions were frequently torn from the terminals and were floating in the vascular space. Some of LDCV were apparently contained in the protrusions. The present findings provided the morphological evidence for the secretion manner from secretory terminals in the PP which was quite similar to apocrine-like structure.**

**Key words**: Large dense core vesicles, Apocrine-like structure, Posterior pituitary, Protrusion, Rat

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## Introduction

The posterior pituitary (PP) is an essential component of the neuroendocrine system and is only a large store of the secretory granules, identified as large dense core vesicles (LDCV), which are synthesized from other organs. Exocytosis is thought to be the sole secretion manner of the secretory granules by which the regulated secretion of stored protein and peptides occurs [1-3]. It is generally accepted that such stored materials are released into the tissue fluid of perivascular space by excytosis and then immediately enter blood circulation through the walls of fenestrated capillaries [4,5]. However, it is of interest that morphology of terminals is changeable, particularly under the direct effects on expression of mRNA. In our previous study of synaptic transport of neuronal marker enzyme in the central nervous system, terminals containing the protein showed apocrine-like structure induced by Rab3A-siRNA (6). In this point, the secretion granules might not be always conformed to the secretion manner of exocytosis although the secretory granules coated with membrane are indicated to exist not only in axon terminals but also occasionally in perivascular space after exocytosis [3,7,8]..

Therefore, in the present study secretion terminals containing LDCV in the rat PP were investigated at the electron microscopic level and secretion manner from the terminals was revalued in detail.

## Materials and methods

16 adult male Wistar rats, weighing 180-230g, were obtained from CLEA Japan, Inc (Tokyo, Japan). The animals were individually housed in a plastic cage under 12-h light and dark cycle (light on from 6:00AM and 6:00PM) in a temperature-controlled room (22±2℃). Experimental procedures were conducted in accordance with National Institute of Health (NIH) for Care and Use of Laboratory Animals. The Kagawa University Animal Care and Use Committee approved the procedures, and all efforts were made to minimize the number of animals used and their suffering.

Animals were anesthetized with intra-peritoneal injection of chloral hydrate (490 mg/kg), and sacrificed by perfusion through the ascending aorta with 0.1 M phosphate buffer (pH 7.4) followed by a fixative of 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1M phosphate

buffer. For electron microscopic observations, the PP was cut transversely into 200 µm-thick sections using a vibratome (Leica VT 1000S, Germany). According to our previous studies (6,9), the sections were postfixed in buffer 1% osmium tetroxide for 2 hours, block-stained in saturated uranyl acetate for 1 hour, dehydrated in a graded ethanol series and embedded in epoxy resin mixture. The region of the PP was identified by examination of toluidine blue-stained 1 µm-thick sections. Ultrathin sections were cut and observed without lead citrate staining using a JEM 200 CX electron microscope in order to compare with the findings of further horseradish peroxidase experiments.

## Results

Toluidine blue-stained 1 µm-thick sections showed various structures in the PP, such as fibers, pituicytes, capillaries and vascular spaces. In the electron microscopic observations, LDCV were recognized easily by containing the electron dense substance when lead citrate staining was omitted. There were a lot of large terminals in which small clear (30-35 nm in diameter) and LDCV (180-230 nm in diameter) were present. However, the morphological changes of LDCV characterized by exocytosis were never found around membranes of terminals (Fig. 1).

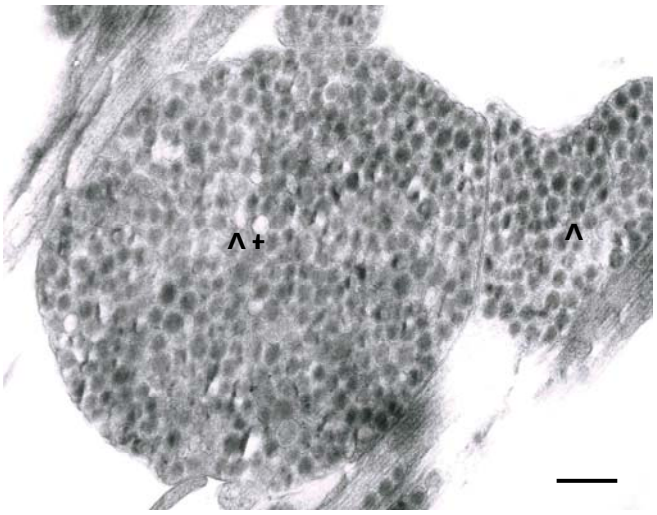

***Figure 1.*** *Electron micrograph of two large axon terminals containing many LDCV and a few vacuoles in the PP. Note that there was no morphological change of LDCV characterized by exocytosis around membranes of terminals. At, axon terminal. Calibration bar = 0.5 µm*

It was of particular interest that terminals occasionally showed protrusions of membranes containing electron dense substance (Figs. 2A and B). The protrusions were frequently torn from the terminals (Fig. 2C) and were floating in the vascular space (Figs. 2E and F). Some of LDCV were apparently contained in the protrusions and occasionally identified as floating substance (Figs. 2E and F). Additionally, membranes of terminals were not always smooth. Protrusions of vacuoles were present around the rugged and blurred edge forming the irregular surface of terminals (Fig. 2D). With respect to the vacuoles, in the present study it was not clear whether the vacuoles are originated from membranes containing LDCV

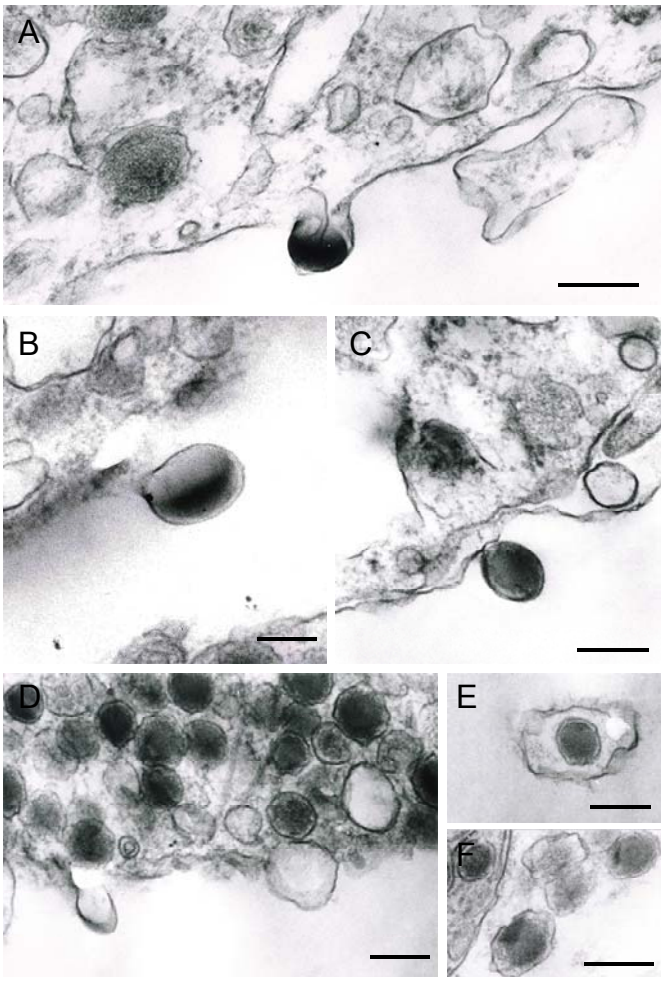


***Figure 2.*** *Electron micrographs of protrusions of membranes containing electron dense substance (A-C), vacuoles (D) and floating of LDCV in vascular space (E and F). Note that electron dense substance coated with the membrane protruded to outside of the axon terminals (A-C) and finally it was frequently torn from terminals (C). Some of LDCV were apparently contained in the protrusions and identified as floating substance (E and F). Membrane of terminals was not always smooth. Protrusions of vacuoles were present around the rugged and blurred edge forming the irregular surface of terminals (D). Arrows indicate vacuoles around surface membrane with rugged and blurred edge. Calibration bars = 0.2 µm in A-F*

## Discussion

The PP was composed of unmyelinated nerve fibers, pituicytes and connective tissues abound in the blood capillaries. It has been long known that nonapeptide hormones arginin-vasopressin and oxytocin are produced in the supraoptic and paraventricular nucleus of the anterior hypothalamus. These neurohormones are transported through hypothalamo-hypophysial pathways and released into the blood vessels of the PP. Release of the arginin-vasopressin and oxytocin contained in the LDCV is considered to be based on not only excitatory transmitter such as glutamate acting through well-established receptors but also a rapid action of oestradiol acting by a mechanism involving NMDA receptors [10].

It was the most conspicuous feature in the present study that LDCV in the secretory terminals are contained in protrusions of the membrane, torn from the terminals and floating in the vascular space. This secretory manner from terminals is quite similar to that of apocrine-like structure which is frequently seen in various organs of the body, such as ovarian follicle cells [11], lung epithelial cells [12] and sweat glands [13]. This phenomenon is considered to be inconsistent with the theory that the coated vesicles retrieve the membrane of the granules have been exocytosed [3,4,7,14]. However, our previous electron microscopic observations apparently indicated the existence of apocrine-like structure of terminals in the central nervous system. In this study synaptic transport of protein in terminals was indicated to be made by forming apocrine-like structure which is particularly induced by Rab3A-siRNA [6].

The present findings provided the morphological evidence for the secretion manner from secretory terminals in the PP which was quite similar to apocrine-like structure.

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