

Identification of the porosome complex in the hair cell

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Abstract

Porosomes are proposed to be the universal secretory machinery of the cell plasma membrane, where membrane-bound secretory vesicles transiently dock and fuse to expel their contents to the extracellular space during cell secretion. In neurons, porosomes are manifested as cup-shaped lipoprotein structures in the presynaptic membrane, 12–17 nm in diameter and possessing a central plug. Hair cells of hearing and balance secrete transmitter from synaptic vesicles in sensory signal transduction, but it has not previously been demonstrated that these mechanosensory cells possess porosome structures that could participate in the secretory process. In the present study, we provide evidence obtained using transmission electron microscopy that porosome structures exist in the hair cell, suggesting a mechanism of hair-cell transmitter secretion markedly different from that of the classic view of the exocytotic process.

Keywords: porosome; hair cell; exocytosis; reeptoneural transmission; ribbon synapse

1. Introduction

Cell secretion is among the most fundamental processes of living cells, playing a central role in cell division, exocrine and endocrine function, and neurotransmitter release. Classically the mechanism for the release of vesicles is thought to involve the fusion of the synaptic vesicles with the cell plasma membrane and eventual incorporation into the membrane. According to this view, the membrane bilayer is later recycled by recreation of vesicles from the cell membrane (Dresbach et al., 2001).

A new structure, the ‘porosome’ has been described, which facilitates vesicular release. The porosome was first discovered in pancreatic acinar cells (Schneider et al., 1997; Cho et al., 2002c; Jena et al., 2003; Jeremic et al., 2003; Elshennawy, 2011). In addition to their identification in acinar cells, porosome structures have been documented in pituitary growth hormone-secreting cells (Cho et al., 2002b), adrenal chromaffin cells (Cho et al., 2002d), β -cells of the endocrine pancreas (Jena, 2004), neurons (Cho et al., 2004, 2008; Siksou et al., 2007) and astrocytes (Lee et al., 2009). The proposed mechanism for porosome function comprises a stable docking assembly that allows the synaptic vesicle to attach, release its contents and then break off to return intracellularly. Support for the porosome-docking mechanism includes data that the observed capacitance changes after exocytosis are less than what would be expected from a pure fusion mechanism (cf. Albillos et al., 1997). Additionally, the number of vesicles present before and after exocytosis can be little changed (Ceccarelli et al., 1973; Cho et al., 2002a; Lee et al., 2004). Key vesicular docking proteins (Ramakrishnan et al., 2009), including target-SNAREs (soluble *N*-ethylmaleimide-sensitive factor-attachment protein receptors) and vesicle-SNAREs, are present at the porosome complex (Jena et al., 2003; Cho et al., 2004). Recent advances in imaging techniques, including AFM (atomic force microscopy), have given greater

understanding of the complexity of this process. The structure of the porosome comprises a stable 8–12 protein umbrella- or cup-shaped transmembrane complex that has multiple conformational states (Cho et al., 2010), depending on whether the porosome complex is resting or in active secretion (Schneider et al., 1997). Transmission electron microscopy has also provided supporting evidence for the porosome at the vesicle and cell membrane interface (Cho et al., 2008).

We have examined the afferent and efferent synapses of a vestibular hair cell of the rainbow trout (*Oncorhynchus mykiss*) to determine whether porosome-like structures are present in this sensory receptor cell. Although the mechanism of synaptic vesicle secretion in auditory and vestibular hair cells is not well understood (Fuchs and Parsons, 2006), the prevailing view is that hair-cell synaptic vesicles undergo exocytosis according to the classic mechanism (Südhof, 1995; Nouvian et al., 2006), and their membranes are re-cycled through the hair cell’s plasma membrane, to be recovered by the process of endocytosis (Ceccarelli et al., 1973). Results of electrophysiological studies showing an increase in hair-cell membrane capacitance after stimulation (Neher, 1998; Spassova et al., 2004) have been interpreted as supporting the classical exocytotic mechanism in the hair cell. However, the possibility of another mechanism for reeptoneural secretion became apparent when we examined transmission electron micrographs of the synaptic structure of saccular hair cells of the trout, as described herein.

2. Experimental

2.1. Electron microscopy

Saccular maculae from rainbow trout were dissected (Drescher et al., 1987a) and transferred to Trump’s fixative consisting of 1%

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Abbreviation used: SNARE, soluble *N*-ethylmaleimide-sensitive factor-attachment protein receptor.

glutaraldehyde, 4% formalin and 0.1 M sodium phosphate, pH 7.2 (McDowell and Trump, 1976). Tissues were post-fixed in 1% osmium tetroxide for 1 h, dehydrated, and embedded in Embed 812 (Electron Microscopy Sciences). Pale gold-to-silver sections (65–70 nm thick) were placed on 200-mesh copper grids, post-stained with aqueous uranyl acetate and Reynolds lead citrate (Reynolds, 1963), examined with a Zeiss EM10-CA transmission electron microscope, and photographed. Electron micrograph photos representing a magnification of $\times 200000$ actual size were quantitatively analysed with Bioquant II software (R & M Biometrics).

3. Results and discussion

3.1. Hair-cell synapses

Figure 1 shows a representative overview of a section of the sensory macula of the trout saccule, the organ of hearing in teleosts. Hair cells (H), labelled in the illustration, contain dense synaptic bodies (B), each surrounded by a halo of clear vesicles (Hama and Saito, 1977). These synaptic bodies with vesicles characterize the excitatory afferent ribbon synapse present in hair cells and other receptor cells (Matthews and Fuchs, 2010), thought to aid in fast synchronous release (Parsons and Sterling, 2003). Two of these ribbon synapses associated with different hair cells are apposed to a single afferent fibre (A in Figure 1). An efferent ending (E) also synapses on the hair cell located in the

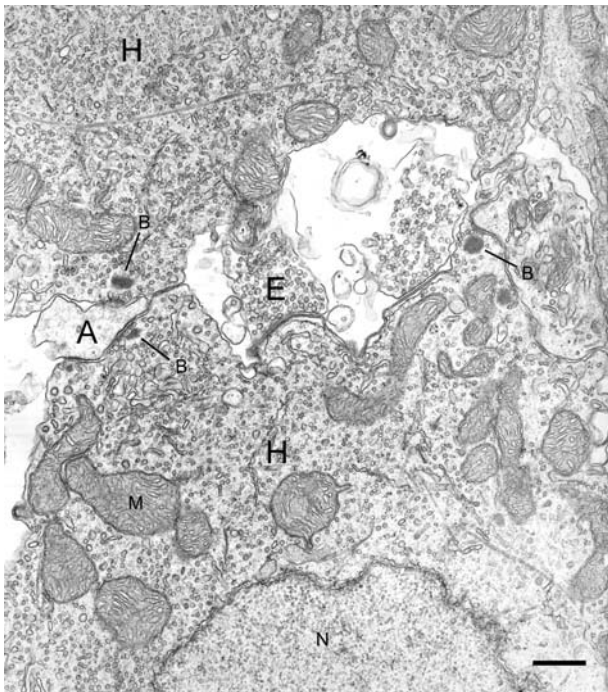


Figure 1 Transmission electron micrograph showing synaptic structures and neural endings of rainbow trout saccular hair cells

Afferent (A) and efferent (E) nerve endings contact the hair cells (H). Synaptic bodies (B) in the presynaptic region of hair cells are indicated with lines. M, mitochondria; N, nucleus. Scale bar=500 nm.

lower portion of the photo. Efferent endings are filled with clear vesicles, and may modify the afferent signal of the teleost saccular hair cell in an inhibitory manner (Furukawa, 1966). In the present work using Trump's fixative, the average diameter of the vesicles encircling synaptic bodies (Drescher et al., 1987b) was 46.4 ± 0.2 nm ($n=640$), whereas the average diameter of efferent vesicles was 53.1 ± 0.3 nm ($n=433$).

3.2. Afferent synapse

At higher magnification, the structure of synaptic vesicles at the hair-cell afferent synapse is shown in Figure 2. Two different, representative docked vesicles comprising putative porosome structures are shown in Figures 2(b) and 2(c). Figure 2(d) (from Figure 2c) outlines the hair-cell vesicle (yellow), including its associated porosome region (orange) and pre- and post-synaptic membranes (yellow). A central plug is indicated by the blue arrowhead. The observed porosome-like structure at the hair-cell synapse in Figure 2 is remarkably similar to that described for neuronal cells (Cho et al., 2004).

3.3. Efferent synapse

Figure 3 shows magnified presumptive porosome structures present in efferent endings synapsing on hair cells. Similar to Figure 2,

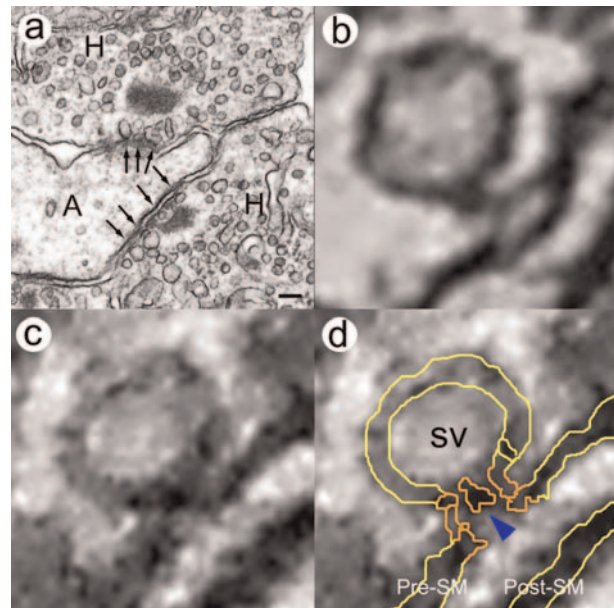


Figure 2 Porosomes at the hair-cell (H) plasma membrane of the afferent synapse in the trout saccular macula

(a) Electron micrograph shows porosome locations (arrows) at the afferent nerve ending, with 40–50 nm synaptic vesicles (SV), synaptic body, and intact presynaptic and postsynaptic membranes. Scale bar=100 nm. (b, c) Two representative porosomes shown at higher magnification. Vesicle diameter is ~ 46 nm, here and in Figures 3(b) and 3(c). (d) Outline of the porosome region from (c). Orange lines designate the 10–12 nm diameter porosome structure observed at the presynaptic membrane (Pre-SM), and yellow lines show an ~ 45 -nm synaptic vesicle docked at the porosome base (base=upper portion of porosome in the figure, the bottom of a 'cup' structure; see Cho et al., 2010) and the postsynaptic membrane (Post-SM). Blue arrowhead indicates the porosome's central plug.

an overview of the synaptic region is shown in Figure 3(a), and two representative views of the porosome structures are presented in Figures 3(b) and 3(c). A coloured outlined version of Figure 3(c) is shown in Figure 3(d). The characteristic porosome configuration is again apparent, including the presence of a central plug (arrowhead).

3.4. Porosomes in a receptor cell

From the results of the current study, there is clear support for the presence of porosome-like structures in the teleost vestibular hair cell. We found these structures many times in the photographs that were examined (results not shown), both for afferent and efferent synapses (24 times for afferent vesicles, 24 times for efferent vesicles).

So far, porosomes have been described in a number of cell types already mentioned (reviewed by Jena, 2009). The present study gives the first description of porosomes in the saccular hair cell and also in a sensory receptor cell. Although more experiments are underway to determine characteristics such as the biochemical composition and membrane properties of hair-cell porosomes, these morphological observations bolster the hypothesis that porosome structures, and their implied fusion mechanism, are universally present in secretory cells.

Author contribution

Won Jin Cho selected the transmission electron micrographs and assembled the illustrations. Marian Drescher prepared and

performed measurements on electron microscopic sections. Dennis Drescher formulated hypotheses and wrote the manuscript.

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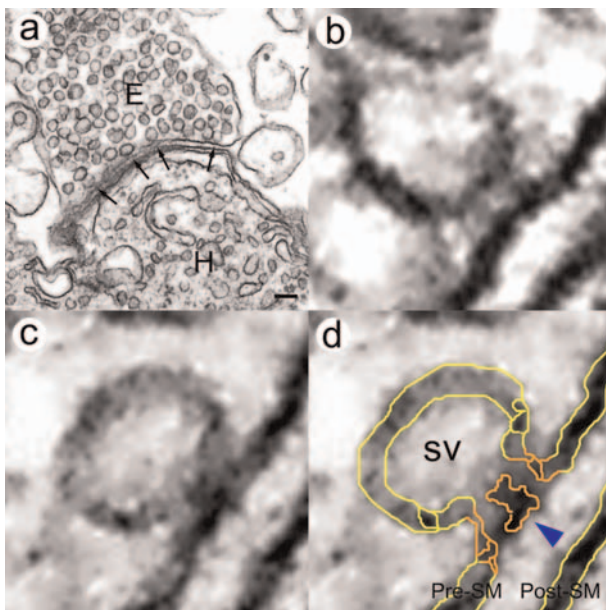


Figure 3 Porosomes at the plasma membrane of an efferent (E) nerve ending synapsing on the trout saccular hair cell (H)

(a) Electron micrograph shows porosomes (arrows). (b, c) Two representative efferent porosomes shown at higher magnification with intact presynaptic and postsynaptic membranes. (d) Outline of the porosome region from (c). As in Figure 2, orange lines designate the porosome structure at the presynaptic membrane. Again, a synaptic vesicle (SV) is docked at the porosome base. The presynaptic membrane (Pre-SM) and postsynaptic membrane (Post-SM) are outlined in yellow. The blue arrowhead indicates the central plug in the porosome.

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