Identification of the porosome complex in the hair cell

Dennis G. Drescher1*,†, Won Jin Cho* and Marian J. Drescher*

1 Department of Otolaryngology, Wayne State University School of Medicine, Detroit, MI 48201, U.S.A.
2 Department of Biochemistry and Molecular Biology, Wayne State University School of Medicine, Detroit, MI 48201, U.S.A.

Cite this article as: Drescher DG, Cho WJ and Drescher MJ (2011) Identification of the porosome complex in the hair cell. Cell Biol. Int. Rep. 18(1):art:e00012.doi:10.1042/CBR20110005

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; receptoneural 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 reformation 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; Sikou 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 receptoneural 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%
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 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 $46.4 \pm 0.2$ nm ($n=640$), whereas the average diameter of efferent vesicles was $53.1 \pm 0.3$ 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,
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.

Funding

This work was supported by the National Institutes of Health [grant numbers DC000156 (to D.G.D.) and DC004076 (to M.J.D.)].

References

Albillos A, Dernick G, Horstmann H, Almers W, Alvarez de Toledo G, Lindau M. The exocytotic event in chromaffin cells revealed by patch amperometry. Nature 1997;389:509–12.

Ceccarelli B, Hurtub WP, Mauro A. Turnover of transmitter and synaptic vesicles at the frog neuromuscular junction. J Cell Biol 1973;57:499–524.

Cho SJ, Cho J, Jena BP. The number of secretory vesicles remains unchanged following exocytosis. Cell Biol Int 2002a;26:29–33.

Cho SJ, Jeflinjka I, Glavaski A, Jeflinjka S, Jena BP, Anderson LL. Structure and dynamics of the fusion pores in live GH-secreting cells revealed using atomic force microscopy. Endocrinology 2002b;143:1144–8.

Cho SJ, Quinn AS, Stromer MH, Dash S, Cho J, Taatjes DJ, Jena BP. Structure and dynamics of the fusion pore in live cells. Cell Biol Int 2002c;26:35–42.

Cho SJ, Wakade A, Pappas GD, Jena BP. New structure involved in transient membrane fusion and exocytosis. Ann NY Acad Sci 2002d;971:254–6.

Cho WJ, Jeremic A, Rognlien KT, Zhvania MG, Lazriserhli I, Tamar B, Jena BP. Structure, isolation, composition and reconstitution of the neuronal fusion pore. Cell Biol Int 2004;28:699–708.

Cho WJ, Ren G, Jena BP. EM 3D contour maps provide protein assembly at the nanoscale within the neuronal porosome complex. J Microsc 2008;232:106–11.

Cho WJ, Lee JS, Jena BP. Conformation states of the neuronal porosome complex. Cell Biol Int 2010;34:1129–32.

Dresbach T, Qualmann B, Kessels MM, Garner CC, Gundelfinger ED. The presynaptic cytomatrix of brain synapses. Cell Mol Life Sci 2001;58:94–116.

Drescher MJ, Drescher DG, Hatfield JS. Potassium-evoked release of endogenous primary amine-containing compounds from the trout saccular macula and saccular nerve in vitro. Brain Res 1987a;417:39–50.

Drescher MJ, Drescher DG, Hatfield JS, Seitz CM. Synaptic morphology and vesicle morphometry of hair cells in the teleost saccular macula. Soc Neurosci Abstr 1987b;13:43.

Elshennawy WW. Image processing and numerical analysis approaches of porosome in mammalian pancreatic acinar cell. J Am Sci 2011;7:835–43.

Fuchs PA, Parsons TD. The synaptic physiology of hair cells. In: Fay RR, Popper AN, editors. Handbook of Auditory Research. New York: Springer; 2006. p. 249–312.

Furukawa T. Synaptic interaction at the Mauthner cell of goldfish. Prog Brain Res 1966;21:44–70.

Hama K, Saito K. Fine structure of the afferent synapse of the hair cells in the saccular macula of the goldfish, with special reference to the anastomosing tubules. J Neurocytol 1977;6:361–73.

Jena BP. Discovery of the porosome: revealing the molecular mechanism of secretion and membrane fusion in cells. J Cell Mol Med 2004;8:1–21.

Jena BP. Functional organization of the porosome complex and associated structures facilitating cellular secretion. Physiology 2009;24:367–76.

Jena BP, Cho SJ, Jeremic A, Stromer MH, Abu-Hamdah R. Structure and composition of the fusion pore. Biophys J 2003;84:1337–43.
Hair-cell porosome

Jeremic A, Kelly M, Cho SJ, Stromer MH, Jena BP. Reconstituted fusion pore. Biophys J 2003;85:2035–43.

Lee JS, Mayes MS, Stromer MH, Scanes CG, Jef tinija S, Anderson LL. Number of secretory vesicles in growth hormone cells of the pituitary remains unchanged after secretion. Exp Biol Med 2004;229:632–9.

Lee JS, Cho WJ, Jef tinija K, Jef tinija S, Jena BP. Porosome in astrocytes. J Cell Mol Med 2009;13:365–72.

Matthews G, Fuchs P. The diverse roles of ribbon synapses in sensory neurotransmission. Nat Rev Neurosci 2010;11:812–22.

McDowell EM, Trump BF. Histologic fixatives suitable for diagnostic light and electron microscopy. Arch Pathol Lab Med 1976;100:405–14.

Neher E. Vesicle pools and Ca²⁺ microdomains: new tools for understanding their roles in neurotransmitter release. Neuron 1998;20:389–99.

Nouvian R, Beutner D, Parsons TD, Moser T. Structure and function of the hair cell ribbon synapse. J Membr Biol 2006;209:153–65.

Parsons TD, Sterling P. Synaptic ribbon: conveyor belt or safety belt? Neuron 2003;37:379–82.

Ramakrishnan NA, Drescher MJ, Drescher DG. Direct interaction of otoferlin with syntaxin 1A, SNAP-25, and the L-type voltage-gated calcium channel Caᵥ1.3. J Biol Chem 2009;284:1364–72.

Reynolds ES. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J Cell Biol 1963;17:208–12.

Schneider SW, Sritharan KC, Geibel JP, Oberleithner H, Jena BP. Surface dynamics in living acinar cells imaged by atomic force microscopy; identification of plasma membrane structures involved in exocytosis. Proc Natl Acad Sci USA 1997;94:316–21.

Siksou L, Rostaing P, Lechaire JP, Boudier T, Ohtsuka T, Fejtova A, Kao HT et al. Three-dimensional architecture of presynaptic terminal cytomatrix. J Neurosci 2007;27:6868–77.

Spassova MA, Avissar M, Furman AC, Crumling MA, Saunders JC, Parsons TD. Evidence that rapid vesicle replenishment of the synaptic ribbon mediates recovery from short-term adaptation at the hair cell afferent synapse. J Assoc Res Otolaryngol 2004;5:376–90.

Südhof TC. The synaptic vesicle cycle: a cascade of protein–protein interactions. Nature 1995;375:645–53.

Received 22 September 2011/accepted 27 September 2011

Published as Immediate Publication 7 October 2011, doi 10.1042/CBR20110005