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Tomographic reconstruction reveals the morphology of a unique cellular organelle, the aggregated macrotubules (Macrotubuli aggregati) of human retinal horizontal cells

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ABSTRACT

Horizontal cells of the human retina contain unique tubular organelles that have a diameter which is about 10 times larger than that of microtubules (~230 nm). These macrotubuli in most cases form regular aggregates. Therefore we propose to introduce them as Macrotubuli aggregati in the Terminologia histologica. Tomographic investigation of the structures revealed that the walls of the tubules most probably consist of intermediate filaments running nearly parallel to each other and show somewhat regularly attached ribosomes on their inner and also outer surface. About 2% of the organelles exhibit double- to multiple layered walls and less than 1% resemble large scrolls. The tubules may extend 10 to over 20 μm in the cytoplasm and are also encountered in soma-near processes extending into the outer plexiform layer. It remains unclear why these structures are only present in humans and few other species and why almost only in horizontal cells. Speculations on possible functions are discussed.

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1. Introduction

Horizontal cells (Hc) of the human retina interconnect a large number of rod and/or cone synaptic terminals and play an important role in signal processing, i.e. they are the morphological correlate for lateral inhibition needed for visual contrast enhancement and movement perception (Sjöstrand, 2003a,b, 2004; Zhang et al., 2011). Their negative feedback to cones by GABA release is important for the formation of centre-surround receptive fields in bipolar and ganglion cells, and thus for normal spatial and chromatic perception (Thoreson and Mangel, 2012; Hanneken et al., 2013). Hc are encountered in the outermost part of the inner nuclear layer of the retina and are connected via their dendrites to cones. Thus, in some cases, Hc even seem to be colour specific (Sandmann et al., 1996). Currently, H1 Hc with large cell bodies, thick radiating dendrites and flower-like dendritic terminal clusters contacting short wavelength (blue) cones and smaller H2 Hc which send their thin meandering dendrites to the remaining majority of red and green cones can be distinguished (Wässle et al., 1989; Dacey et al., 1996; Zhang et al., 2011). Immunohistochemically H1, but not H2 horizontal cells, express protocadherin subunit β16 (Puller and Haverkamp, 2011) whereas only H2 cells are positive for calbindin (Wässle et al., 2000). Kolb et al. (1994) described a third type of horizontal cells in human retina, the H3 cells which resemble H1 cells. However, the heavyset H3 cells show larger and more asymmetrical dendritic trees and ~20% of them show a descending process to the outer strata of the outer plexiform layer (Kolb et al., 1994).

In human or primate retina and a very few other species light microscopic investigation reveals rod-like basophilic structures (Fig. 1) in Hc of the uppermost inner nuclear layer extending into the outer plexiform layer (OPL). They were first described by Kolmer (1918) as 18 μm long and 1–2.5 μm thick bodies, with stump ends resembling crystals he called "kristallstäbchenförmige

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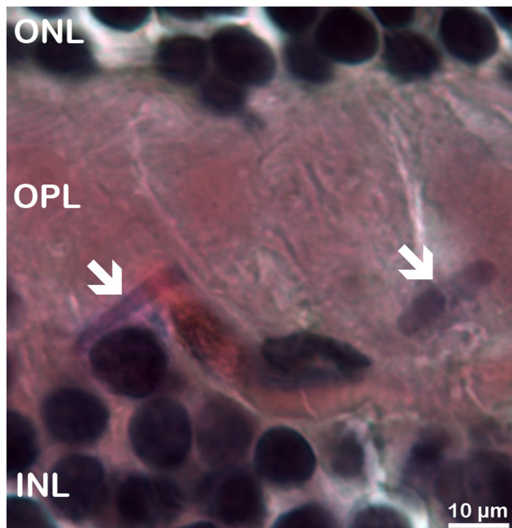

Fig. 1. Light microscopic view of a "Kolmer's crystalloid" (arrows) which is a larger aggregation of macrotubules with attached ribosomes. Note the considerable length of the basophilic stained structure at the border of the inner nuclear layer (INL) to the outer plexiform layer (OPL) of the freshly fixed retina of a 62 year old human male.

Gebilde". Therefore, the term Kolmer's crystalloid was established. Ultrastructurally, however, Missotten (1961) was the first one to show that these crystalloids are tubules (length ~10–20 μm, diameter ~200 nm). Since this diameter is about 10× larger than that of microtubules and due to the fact that the tubules typically form regular aggregates we propose to term them Macrotubuli aggregati (MA).

Originally MA were discovered microscopically by Dogiel in 1884 but mistaken as a tangential cut flat major cell process or as ridges along the cell surface caused by pressure of neighbouring cell processes (Dogiel, 1891). Kolmer (1918) was the first investigator who realised that darkly stained stripes he noted in human horizontal cell cytoplasm had to be something special. They reminded him of Reinke's crystalloid of Leydig cells and therefore he also termed them as crystalloids. Application of different acid or basic staining agents showed him that their staining resembled that of protein (Kolmer, 1930a). But not everybody accepted Kolmer's view, e.g. Polyak (1941) just described them as a peculiar structure in the inner zone of the OPL with a small spherical shaped body and few dendrites spreading in horizontal direction. He could not decide whether this was caused by accidentally stained parts of already known neurons swollen into varicosities and simulating unknown cells or simply by displaced horizontal cells. Walls (1939) even claimed MA to be of lipoid material representing always staining artefacts.

MA are very rare and have only been encountered at the following locations: Endothelial cells of the human cornea (four cases: 3× corneal dystrophy, 1× hyperparathyroidism; Jensen et al., 1975) where the authors considered it to be a special form of RER. However, MA are not present in normal human corneal endothelium, as confirmed in the material investigated in this study.

MA were depicted by Kappe (1992a,b, 1993) in pinealocytes, so far only in the common spiny mouse (*Acomys cahirinus dimidiatus*). In our (unpublished) investigations on pinealocytes of mouse, rat, guinea pig and humans these organelles were never observed. Further, and more important, MA have been shown to be present in the cytoplasm of horizontal cells of the retina of humans (Kolmer, 1918, 1936; Villegas, 1961; Hogan et al., 1971; Uga et al., 1969), adult pedigree Beagle dogs (*Canis lupus familiars*; Fujii et al., 1976), monkeys, but only chimpanzee (*Pan troglodytes*; Kolmer, 1930a,b), red guenon (*Erythrocebus patas*; Gallego, 1971) and rhesus macaque (*Macaca mulatta*; Gallego, 1971), but the latter is questionable since Kolmer (1918) did not find MA in this species.

The tubular MA-like structures were searched for, but not found in retinae from tortoise carp, shark or ray (Yamada and Ishikawa, 1965) and a variety of monkeys (*Macaca sinius*, *M. cynomolgus*; Kolmer, 1918), 25 other primate species including orangutan, gibbon (Kolmer, 1930a), *Macaca spec.*, other old world apes, *Ateles spec.*, *Hapale spec.*, other new world apes, anthropoids and semi-apes such as lemurs (Kolmer, 1930b). In newborn humans, however, MA were not detected (Kolmer, 1918).

Some investigators claimed that the walls of the MA tubules consisted of membranes only (Missotten, 1961, 1964, 1965; Yamada, 1966), indeed Jensen et al. (1975) even postulated a double unit membrane. Others proposed that MA consist of ordered filaments (Kappe, 1992a,b, 1993; Uga and Ikui, 1969; Uga et al., 1969). To finally clarify this, an investigation applying very higher resolution TEM and tomographic serial section reconstruction methods like those of Ladinsky et al. (1999) are required. Thus the present investigation was undertaken to perform three dimensional (3D) analysis of these extraordinary cell organelles and to assess the quality of the 3D reconstruction obtained from tilted images from several different state-of-the-art electron microscopes.

2. Materials and methods

2.1. Material

The tissue blocks used in this study were derived from retinae of three different adult human males. All samples were obtained in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. Sample A was kindly provided by a 62 year old patient with a malignant melanoma of the right choroidea. It was obtained at the operation theatre of the University eye clinic Mainz, Germany. Samples B and C originated from two different male multiple organ donors (62 and 81-years old). These preparations were obtained from enucleated bulbs from which the cornea was removed for transplantation by Prof. Ardjomand and Prof. Haller-Schober, Dept. of Ophthalmology, Karl-Franzens-University Medical School, Graz, Austria. All material for tomographical analysis was dissected from tiny non-central areas of the retina, in the tumour eye (Sample A) at a distance of over 10 mm from the visible tumour. Corneal endothelium was investigated using the cornea of the ocular bulb belonging to Sample A and the eye of a Wistar rat used for other investigations, as a control animal.

2.2. Methods

The sample A was fixed immediately after the enucleated bulb was dissected (not later than 4 min after blood supply had stopped) in a modified Karnovsky (1965) solution of 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M PBS (phosphate buffered saline) and stored at 4 °C overnight. Some small pieces into which the retina was dissected were transferred into a buffered formalin solution for routine HE staining and light microscopic investigation. All remaining tissue pieces were used for electron microscopy and washed in 0.1 M PBS, pH 7.4, with 6.8% sucrose, post-osmicated for 90 min (2% OsO_4 in 0.1 M PBS, pH 7.4), block-stained in 0.5% uranyl acetate plus 1% phosphotungstic acid in 70% acetone overnight, dehydrated in acetone and embedded in Epon^{TM}.

Samples B and C were obtained from eye bulbs stored several weeks in the same fixative and then treated similarly. However, fixation of these samples, did not start until 4 h after death.

Ultrathin sections were cut with a diamond knife (Diatomé, Biel, Switzerland) on a Reichert UltracutE ultramicrotome

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