Functional Properties of Photoreceptors in Cultured Human Retina

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Research question and background

Retinitis pigmentosa is an eye disease that leads to progressive loss of vision and ultimately blindness. In this disease photoreceptors lose their lights sensitivity and finally die. We investigate whether we can use the novel technique optogenetics to restore the light zsensitibity of photoreceptors and thus restore vision in these patients. Optogenetics makes use of light sensitive proteins such as halorhodopsin and channel rhodopsin. Cell-specific expression of these proteins will make these cells light sensitive. Recently optogenetics successfully restored light sensitivity to degenerated retinas of mutant mice and to ex vivo human retina (Busskamp et al, 2010). To assess to what extent functional is restored, and to determine the optimal light stimuli needed, we have been recording neuronal responses from cultured human retina explants. As a first step we assessed the quality of the cultured human retinal explants by studying the expression patters of cellular markers of key cells in the retina. Secody the basic conditions of cones in cultured human retinal explants by examining their ionic currents.

Methods and tissues used

Human eyes were obtained from the Euro Tissue Bank and the Netherlands Brain Bank in Amsterdam. The isolated retinas were put into culture less than 30 hours post-mortem at 37^{o}C on polycarbonate membranes. They were cultured for up to 10 days. The retinas were fixed at room temperature in 0.2M phosphate-buffered (pH 7.4) 4% paraformaldehyde for immunohistochemistry experiments, and stained with the next antibodies: rabbit anti opsin red/green 1:100, rabbit anti opsin blue 1:100, rabbit anti rhodopsin 1:100, Protein Kinase C-alpha (PKC-α) 1:200, Ribeye 1:500, Go-α 1:1000, cleaved caspase-3 1:250, mGluR6 1:5000. For electrophysiological analysis, retinal explants were transferred to a recording chamber continuously perfused with 37^{o}C Ames medium (pH 7.8) and currents recorded by whole-cell voltage clamp techniques.

Results and conclusion

Immunohistochemistry staining of the fixed ex vivo cultured human retinas showed a clear labeling of the antibodies for until 10 culturing days. Cultured human retina explant cones expressed Calcium (I_{Ca}), Calcium activated Chloride ($I_{Cl[Ca]}$), Sodium (I_{Na}) and Hyperpolarization-activated (I_h) currents. I_{Ca} and $I_{Cl[Ca]}$ have not been previously described for human photoreceptors. I_{Ca} activates at -40mV, peaks at -20mV, and runs down over time. $I_{Cl[Ca]}$ slowly activated when Ca^{2+} flowed into the cell and results in a slow tail current.

The immunocytochemical analysis shows that the structure of the cultured human retinal explants stays intact during the culturing periods up to 10 days. I_{Ca}, $I_{Cl[Ca]}$, I_h and I_{Na} were, respectively, qualitatively similar to L-type Ca^{2+} and $I_{Cl[Ca]}$ found in freshly isolated retinas of other animals and I_h and I_{Na} found in dissociated photoreceptors from fresh human retina explants (see Kawai et al 2005). This suggests the post mortem times and extended culturing did not adversely affect the photoreceptors and that they still make part of an intact retinal system.