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*Spatial variations in the membrane
properties of differentiating fibre
cells isolated from the rat lens*

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**A thesis submitted in fulfilment of the requirements
for the degree of Doctor of Philosophy
The University of Auckland, 2006**

Abstract

The ocular lens is a dense avascular structure composed of a bulk of terminally differentiated elongated fibre cells. During differentiation, lens fibre cells lose their nuclei and organelles and are laid down in successive layers to create a pseudocrystalline cellular architecture, where extracellular space dimensions are kept below the wavelength of visible light. Disruption of this precise structural arrangement leads to the development of cataract and eventual blindness. Differences in membrane transport within different regions of the lens, connected by gap junctions, are thought to generate a unique microcirculatory system which is essential for lens transparency - regulating tissue volume and ionic homeostasis and supplying internalised fibre cells with metabolic and antioxidant support. While the spatial differences in membrane transport thought to drive the lens internal circulation system have been inferred from intact-lens measurements, they have not yet been confirmed at the cellular level.

Problems isolating and working with lens fibre cells has lead to a historical lack of information about the role of these cells in lens physiology. This thesis describes the development and characterisation of a viable isolated fibre cell preparation, representing the first investigation of physiological membrane behaviour in single fibre cells isolated from the rat lens. It was found that by blocking non-selective cation channels activated by fibre cell dissociation with ionic gadolinium, isolated fibre cells of a range of lengths could be maintained viable for several hours in the presence of physiological $[Ca^{2+}]_o$. Furthermore, fibre cell length *in vitro* was similar to that measured in the intact lens, allowing the spatial localisation of measured membrane properties within the outer lens cortex. The measured membrane properties of isolated fibre cells indicate that membrane conductances are altered during fibre cell differentiation, thus creating spatial differences in membrane transport across the lens radius. The measured transition from K^+ -selectivity towards Na^+/Cl^- selectivity during fibre cell elongation confirms inferred properties derived from intact-lens studies. These alterations in the membrane properties of differentiating fibre cells are arranged spatially in a manner appropriate for the generation of the circulating ion fluxes thought to form the lens internal circulation system.

To investigate the role of fibre cell membrane properties in both physiological and pathological circumstances, modulation of fibre cell membrane properties was demonstrated by osmotic and pharmacological means. Data obtained indicates that fibre cells respond to osmotic or metabolic insult by altering their membrane behaviour. The physiological and pathological alteration in fibre cell membrane properties described directly answers significant outstanding questions in lens physiology, and has the potential to generate new therapeutic interventions in the development of cataract

Acknowledgements

This PhD thesis represents the culmination of more years of work than I like to think about. Along the way there have been trials of intellectual and emotional strength, tests of perseverance, and the gradual reconfirmation of a lifelong commitment to science. This realisation has come to me by some of the least-trodden but most scenic of roads, assisted by a series of unique opportunities for which I have innumerable people to thank.

Firstly, my supervisor and mentor, Associate Professor Dr Paul Donaldson. Without endless patient support and encouragement from the Chief, there would be no thesis. It's been a long road, but I think the results now can finally speak for themselves. To the other members of the Molecular Vision Laboratory, thank you for your many and various contributions to making everyday life interesting and stimulating. Your aid and input has been greatly appreciated, as has the opportunity to work alongside you all. In the Department of Physiology, I would like to thank Bruce Smaill, Denis Loiselle, and the other Principal Investigators for making available their time and expertise. To the remainder of the Department of Physiology, academic, research, and support staff, all of you have touched this work in some way - through countless useful discussions, assistance, and scroungings. Thanks are especially due to Arthur and Louey in the workshop, Viv for tireless IT support, and the Teaching Lab staff who look after all of us so well.

To the succession of international scientists who have given me the opportunity to visit and work within their laboratories, you have opened my mind to new experiences and information both within and outside the scope of pure science. I am grateful and humbled to have been able to participate in the ground-breaking research areas being simultaneously tackled around the world by some of the worlds finest research teams. Cheers, vielen Dank, and thank you very much.

As well as a journey of scientific discovery, these last years have been a time of great personal growth. Without the support of my family and friends, I would surely have fallen by the wayside. Your love, comfort, generosity and tolerance have been a staff to support me, and your encouragement and enthusiasm have inspired me through times of hardship. Anyone who has cared enough about me to get even this far through the thesis probably deserves a specific mention. I thank you for your support.

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