2004

Regulation of tissue oxygen levels in the ocular lens

Richard McNulty
University of Wollongong

Regulation of Tissue

Oxygen Levels in the Ocular Lens

A thesis submitted in partial fulfilment of the

requirements for the award of the degree of

Doctor of Philosophy

from

University of Wollongong

by

Dr Richard McNulty

B.A., M.B., B.S. (Syd.)

Department of Chemistry

2004

The only use discovered of this work was that

it may help to make a vain man humble

-after Benjamin Franklin

Certification

I, Richard McNulty, declare that this thesis, submitted in partial fulfilment of the requirements for the award of Doctor of Philosophy, in the Department of Chemistry, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualifications at any other academic institution.

Richard McNulty

6 August 2004

Table of Contents

List of Figures and Tables

Acknowledgements

Thanks to my family for long distance encouragement and support. During this thesis I learnt the true meaning of the phrase "the scientific community." As such, I feel sole authorship is a generous and misleading interpretation of this PhD and I have many people to thank. Numerous colleagues have contributed in various ways to the realization of experiments: Beryl Ortwerth helped with HPLC ascorbate measurements, Richard Mathias and Huan Wang collaborated on the mathematical model, Frank Giblin, Alec Salt and Shane Hale provided guinea pigs, and David Beebe donated human lenses. Helpful advice came from Joseph Bonanno, James Dillon, Tony Hulbert, Ying Bo Shui and Abraham Spector. The Heartland Lion's Eyebank team, especially Kelly Green-O'Neal are thanked for special effort (and cake). Thanks to the team at Trenton Processing, Illinois, for more eyeballs than I can count. Stephen Turney assisted with 2-photon microscopy. Thanks to my lab mum Peggy Winzenburger and to Anna Zandy, my favourite American.

Special thanks must go to Roger Truscott, my long suffering and long distance supervisor. And lastly, but most importantly, to Steven Bassnett, my mentor, friend and stylist, who took a gamble and ensured that the limiting factor in this work was not lack of opportunity or resources: to you, Sire, my humble thanks[1].

---

[1]However, late night deliveries of fish and chips were somewhat lacking.

Publications

Sections of the work described in this thesis have been reported in the following publications:

1. S Bassnett and R McNulty (2003). The effect of elevated intraocular oxygen on organelle degradation in the embryonic chicken lens. J Exp Biol 206, 4353-4361.

2. Richard McNulty, Huan Wang, Richard T Mathias, Beryl J Ortwerth, Roger J W Truscott and Steven Bassnett. Regulation of tissue oxygen levels in the mammalian lens. J Physiol, 2004 (*in press published online July 22, 2004 as 10.1113/jphysiol.2004.068619*).

Abstract

Age-related nuclear cataract is a major cause of blindness and is thought to result from oxidation of key cellular components. Molecular oxygen (O_2) is a possible oxidant in this process. The aim of this thesis was to investigate the regulation of tissue O_2 levels in the ocular lens. We mapped the distribution of O_2 within isolated lenses using a fluorescent optode. All lenses examined (bovine, rabbit and human) showed U-shaped Po_2 profiles across both the optic and equatorial axes resulting in core hypoxia. When equilibrated in physiological conditions (Po_2 environment of 36mmHg), the lens nucleus Po_2 was 1.6 ± 0.5 mmHg (n=6), 12.5 ± 2.5 mmHg (n=6), and 13.8 ± 7.6 mmHg (n=24 pairs) in the bovine, rabbit and human lens, respectively.

We examined a possible role of Po_2 gradients on the development of the embryonic chicken lens. The lens contains two populations of fibre cells, differentiating fibers (DF) in the outer cortex that contain a full complement of organelles (including mitochondria), and mature fibers (MF) in the lens core that do not. Incubating chicken embryos in hyperoxic conditions resulted in larger lenses and an increase in the depth of the DF population compared to lenses from embryos raised in normoxia. This is consistent with the hypothesis that hypoxia triggers the elimination of organelles from the lens.

The consumption of O_2 by the lens was region-dependent. Both respirometric measurements (performed on dissected regions of the bovine lens) and a diffusion/consumption mathematical model showed that approximately 90% of O_2 consumption occurred in the cortex, the remainder occurring in the core which contains only MF. The distribution of mitochondria in the DF layer was mapped using 2-photon or confocal microscopy on living lenses treated with mitochondria-specific dyes (rhodamine

123 or Mito Tracker) and in fixed tissue by immunofluorecence using an antibody to cytochrome *c*-oxidase. The depth of the mitochondria-containing cell layer varied in different species, being 500-760 μm in the bovine lens and 50-100 μm in the human. The Po_2 gradients in the lens extended beyond the boundaries of the mitochondria-containing DF layer, consistent with a role for non-mitochondrial O_2 consumption (albeit a minor one) in the MF region in the intact lens. We therefore examined both mitochondrial and non-mitochondrial processes which may be responsible for modulating lens O_2 levels. Mitochondria-inhibition studies showed that the contribution of oxidative phosphorylation to bovine lens QO_2 was approximately 90%. Interestingly, mitochondria-inhibiting drugs and age had an insignificant effect on human lens QO_2. Ascorbate, found in high levels in the lens, was investigated as an O_2 consumer. Bovine and human lens core O_2 consumption could be induced by metals and inhibited by the metal-chelator DETAPAC, characteristics which are consistent with ascorbate-dependent O_2 consumption. We found no evidence for a role for a trans-plasma membrane oxido-reductase system or photo-oxidation as non-mitochondrial O_2 consumers.

The movement of O_2 through the lens tissue was studied by decreasing lens QO_2. Sub-physiological temperatures resulted in a marked decrease in lens QO_2 and a concomitant flooding of the lens core with O_2. The rapid rise in lens Po_2 at lower temperatures may be of clinical relevance. During vitrectomy, cool, O_2-rich solutions are infused into the eye. We hypothesise that rising lens core O_2 levels during vitrectomy are the cause of the strikingly high incidence of post-vitrectomy nuclear cataract. We have initiated a clinical study to examine whether the use of warmed solutions during surgery can decrease the incidence of post-vitrectomy cataract.

Abbreviations

Abbreviation	Name
±	plus or minus
3D	three-dimensional
α	Bunsen or solubility coefficient (gas volume at STP absorbed per unit volume of liquid)
AAH	artificial aqueous humour
ADP	adenosine diphosphate
ATP	adenosine triphosphate
AREDS	age-related eye disease study
ARNC	age-related nuclear cataract
BSA	bovine serum albumin
CCCP	carbonyl cyanide m-chlorophenylhydrazone
CCD	charge-coupled device
COX	cytochrome *c*-oxidase
$\Delta\Psi_m$	inner mitochondrial membrane potential
DAB	diaminobenzidine
DF	differentiating fibres
D	dioptre
DETAPAC	diethylenetriaminepentaacetic acid
Do_2	diffusion coefficient of O_2
DTPA	diethylenetriaminepentaacetic acid

E	embryonic day
EDTA	ethylenediaminetetraacetic acid
EPR	electron paramagnetic resonance
fMRI	functional magnetic resonance imaging
λ	length constant for O_2 consumption (mm)
G	gauge
GSH	glutathione, reduced
GLUT	glucose transporter
GSSG	glutathione, oxidised
Hb	haemoglobin
hif	hypoxia inducible factor
IgG_{2a}	immunoglobulin G_{2a}
K_m	Michaelis constant
λ	length constant
Mb	myoglobin
MF	mature fibres
mmHg	millimeters of mercury (a unit of gas tension or partial pressure)
MPA	metaphosphoric acid
mRNA	messenger ribonucleic acid
mtDNA	mitochondrial deoxyribonucleic acid
n	number of samples
NAD(P)H	nicotinamide adenine dinucleotide (phosphate), reduced

NMR	nuclear magnetic resonance
NMWL	nominal molecular weight limit
NPA	3-nitropropionic acid
OFZ	organelle-free zone
Pa	Pascal (SI unit of gas tension or partial pressure)
PC	personal computer
Po_2	partial pressure of oxygen or oxygen tension
PBS	phosphate-buffered saline
PET	positron-emission tomography
PMOR	plasma-membrane oxido-reductase system
PMRS	plasma-membrane redox system
ppm	ppm
QO_2	oxygen consumption rate
ROS	reactive oxygen species
RT	real time
SD	standard deviation
STP	standard temperature and pressure (273.15 K or 0°C and 101.325 kPa, 760 mmHg or 1 atmosphere)
SVCT	sodium-dependent vitamin C-transporter
τ	time constant for O_2 consumption (s)
TRIS	tris(hydroxymethyl)aminomethane
UV	ultra-violet

VEGF	vascular endothelial derived growth factor
w/v	weight/volume