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Effect of metabolic insult on amino acids in the ciliary epithelium, lens, and retina

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Abstract

Ischaemic insult deprives ocular tissue of oxygen and essential metabolites leading to anatomical and functional changes. One way to gauge the effect of ocular ischaemia is to determine changes in amino acids likely to be modified due to the intricate interrelationship between cellular metabolism and these compounds. Such an approach, using quantitative silver-intensified immunogold detection of a range of immunoglobulins, can provide a metabolic profile of cells (metabolome). Despite the known alterations in the ciliary epithelium and lens function secondary to ocular ischaemia, little is know about the metabolic profile in both normal ocular tissue and in ocular tissue exposed to metabolic insult.

The major goal of this thesis was to investigate the metabolome of the ciliary epithelium and lens under normal and ischaemic conditions, in parallel to that of the retina, with complete spatial and single cell resolution. The results showed that a glutamate/glutamine cycle exists between the ciliary epithelium and lens, in analogy to the metabolic relationship between neurons and glia in the retina. With the localisation of glutamine synthetase and glutamate transporters, amino acids including glutamate and glutamine accumulate in the ciliary epithelium for secretion, so that lens amino acid levels can be sustained. Experimental manipulations reported in this thesis include enzyme inhibition and exposure to acute ischaemia, both leading to fluctuations of the amino acid levels/distributions in the ciliary epithelium and retina, and changes in amino acids associated with cellular metabolism. In the ischaemia/reperfusion experiments, despite recovery of anatomical features, there were persistent metabolic changes in the ciliary epithelium after four days reperfusion, which results in lower amino acids both in the ciliary epithelium and lens. Further, studies of glutamate transporters led to the discovery of a unique mechanism of glutamate release involving the cystine-glutamate transporter at the photoreceptor ribbon complex. In conclusion, the results offer a parsimonious explanation for the common association of altered ciliary body function and cataract secondary to ischaemic insult and provide evidence for highly conserved amino acid metabolic pathways within ocular tissues.

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Common Abbreviations

AAT	Aspartate aminotransferase
AGB	agmatine
Ala	Alanine
ALT	Alanine aminotransferase
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
APV	2-amino-5-phosphonovaleric acid (selective NMDA receptor antagonist)
Arg	arginine
Asp	aspartate
ATP	adenosine triphosphate
ChAT	choline acetyltransferase
CPPG	(RS)-α-cyclopropyl-4-phosphonophenylglycine (mGluR6 antagonist)
Cx	connexin
DAB	3,3'-diaminobenzidine
EAAT	excitatory amino acid transporter
EPON	epoxy resin
ER	endoplasmic reticulum
FADH2	flavin adenine dinucleotide
GABA	gamma aminobutyric acid
GABA-T	GABA transaminase
GAD	glutamate decarboxylase
GDH	glutamate dehydrogenase
Gln	glutamine
Glu	glutamate
Gly	glycine
GS	glutamine synthetase
GSH	glutathione
iGluR	ionotropic glutamate receptors
INL	inner nuclear layer
IOP	intraocular pressure
IPL	inner plexiform layer
KA	kainate
L-AP4	L-2-amino-4-phosphono-butyric acid (mGluR6 agonist)
mGluR	metabotropic glutamate receptor
MSO	methionine sulfoximine
NADH	nicotinamide adenine dinucleotide

NMDA	N-methyl-D aspartate
NPE	non-pigmented epithelial
OPL	outer plexiform layer
PAG	phosphate-activated glutaminase
PKCα	protein kinase Cα
PE	pigmented epithelial
RPE	retinal pigment epithelium
SHMT	serine hydroxymethyl transferase
SSAD	succinate semi-aldehyde dehydrogenase
Tau	Taurine
TCA cycle	tri-carboxylic acid cycle
Xc-	cystine/glutamate transporter
α-AIB	α-amino isobyric acid