Neurochemical and functional characterization of the ischaemic/reperfused retina

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Author's statement

The experiments carried out within this thesis are entirely my original work. Where other materials, or another person's work has been used due acknowledgement has been made within the main body of the thesis. The thesis is less than 100,000 words, exclusive of tables, figures and bibliography.

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Abstract

Ischaemic cell death has been implicated in a number of retinal diseases, including glaucomatous neuropathy, proliferative diabetic retinopathy and a range of vascular diseases. The cascade of events leading to cell death involves both cellular metabolic changes and a functional component. However, it is yet unknown how long these changes persist, whether all cell classes are affected, and the characteristics of recovery. Moreover, there have been few studies correlating the neurochemical changes with the ensuing functional changes.

The aim of this thesis was to track the metabolic and functional recovery of the ischaemic rat retina, given the premise that: (1) amino acid neurochemistry reflects metabolic integrity and cellular identity, and; (2) the permeation of a cation channel probe called agmatine reflects channel functionality. Quantitative pattern recognition analysis of overlapping amino acid and agmatine expression profiles were used to provide a statistically robust classification of cells according to metabolic and functional characteristics. This classification was spatially complete and with single cell resolution. Finally, the electroretinogram was used to also assess retinal function and corroborate the observed neurochemical changes. These measures were taken at intervals for up to two weeks of reperfusion.

The results show that by 48 hours of reperfusion, amino acid metabolism had returned to near normal levels, although cell classes were missing, and there was persistant cation channel gating anomalies. Immunocytochemical labeling identified a preferential loss of cone bipolar cells, with all remaining rod bipolar cells showing increased cation channel gating. The electroretinogram and agmatine experiments showed that this dysfunction is likely due to abnormal glutamate release from pre-synaptic photoreceptors, detected by changes in post-synaptic agmatine permeation, and not due to the presence of anomalous metabotropic glutamate receptors. Cholinergic amacrine cells demonstrated persistant neurochemical labeling, but did not show cationic flux following stimulation by glutamate agonists. In conclusion, the retina shows remarkable recovery in the amino acid metabolism, although functional changes persist. Finally, structural integrity or immunocytochemical labeling does not necessarily imply that cells maintain functional receptors, or that neurotransmitter release is normal secondary to disease.

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List of abbreviations

a	maximum amplitude (μV) of the gabor that describes the oscillatory potentials
α-KG	α-ketoglutarate
AAT	aspartate aminotransferase
AGB	1-amino-4-guanidobutane; agmatine
ALT	alanine aminotransferase
AMPA	a-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid
APB	2-amino-4-phosphobutyric acid
ATP	adenosine tri-phosphate
Acetyl CoA	acetyl coenzyme A
BCAT	branched chain aminotransferase
BSA	bovine serum albumin
cGMP	cyclic GMP; guanosine 3', 5'-cyclic monophosphate
ChAT	choline acetyl-transferase; labels cholinergic amacrine cells in the rat retina
CNS	central nervous system
ERG	electroretinogram
GABA	γ-aminobutyric acid
GABA-T	γ-aminobutyric acid transferase
GAD	glutamate decarboxylase
GDH	glutamate dehydrogenase
GDP	guanosine di-phosphate
Goα	Go-the alpha subunit; labels ON bipolar cells in the rat retina, including the ON cone and ON rod bipolar cells
GS	glutamine synthetase
GTP	guanosine tri-phosphate
h	frequency (Hz) of the gabor describing oscillatory potentials
i / I	flash intensity (cd.s/m^2) of the PIII / or PII
IgGs	immunoglobulins
iGluR	ionotropic glutamate receptor
K	sensitivity (log cd.s/m^2) of the PII; intensity of the stimulus at half V_{max}

KA	kainic acid
LDH	lactate dehydrogenase
m	peak time (msec) of the gabor that describes the oscillatory potentials
mGluR	metabotropic glutamate receptor
mGluR6	the type 6 metabotropic glutamate receptor; expressed in ON rod and ON cone bipolar cells of the rat retina
NADH	nicotinamide adenine dinucleotide (reduced form)
NMDA	N-methyl-D-aspartate
nSTR	negative scotopic threshold response
OAT	ornithine aminotransferase
ON cell	
OFF cell	
OPs	oscillatory potentials: oscillating wavelets on the rising phase of the b-wave
PII	the rising phase of the b-wave, after Granit (1933)
PIII	(fast PIII), the leading edge of the a-wave, after Granit (1933)
PAG	phosphate activated glutaminase
PDE	phosphosdiesterase
PKCα	protein kinase C the alpha subunit; labels rod bipolar cells in the rat retina
PNR	proximal negative response
pSTR	postitive scotopic threshold response
PV	parvalbumin; labels for AII amacrine cells in the rat retina
Rm_{PIII}	PIII maximum amplitude (μV)
σ	semisaturation constant
s	spread (ms) of the gabor that describes the oscillatory potentials
S	sensitivity ($m^2.cd^{-1}.s^{-3}$) of the PIII; a constant that scales stimulus luminous energy and accounts for the multiple stages of amplification during phototransduction
SSAD	succinate semi-aldehyde dehydrogenase
STR	scotopic threshold response
t	time (sec) after flash onset of the PIII
T	transducin
TCA	tri-carboxylic acid cycle

t_d	delay (ms) to the onset of phototransduction
V_{max}	PII maximum response amplitude (μV)

Single letter notation for the amino acids used

E	glutamate
γ	GABA
G	glycine
D	aspartate
Q	glutamate
A	alanine
T	taurine