DOCTOR OF PHILOSOPHY

Investigation of BACE1 as a stress-induced regulator of neuronal metabolism

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Candidate's Declaration

I hereby declare that all results described in this thesis, unless otherwise stated, are entirely my own work. I further state that the composition of this thesis was performed by myself and none of the material has been submitted for any other degree. Lastly, I verify that all sources have been appropriately cited. The work was carried out in the Medical Research Institute, University of Dundee, under the supervision of Professor M. L. J. Ashford.

John Alexander Findlay

Supervisor's Declaration

I certify that John Alexander Findlay has completed 8 terms of experimental research and has fulfilled the conditions of Ordinance 39, University of Dundee, such that he is eligible to submit the following thesis in application for the degree of Doctor of Philosophy.

Professor M. L. J. Ashford

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Summary

Alzheimer's disease (AD) is the most common cause of dementia, accounting for around 60-70% of cases. AD encompasses large-scale neuronal loss, resulting in progressive memory and other cognitive decline. Presently, there is no cure for dementia and in light of the ageing population demographic, this represents a clear unmet medical and socioeconomic challenge Worldwide. Much of the current AD research focuses on studying the brain once hallmark amyloid-β (Aβ) plaque and neurofibrillary tangle pathologies have presented. However their appearance is extremely end stage and to date, any therapeutic interventions aimed at alleviating them having failed to halt symptoms progression. It may therefore be beneficial to look for earlier changes, with metabolic and oxidative stress events as well as reduced cerebral metabolism thought to occur early on in disease progression.

Evidence from rare, familial AD cases suggests a causative role for Aβ in AD pathogenesis. For this reason, the enzyme β-site amyloid precursor protein (APP) cleaving enzyme 1 (BACE1), the rate-limiting step in Aβ production is currently of great therapeutic interest. With the prevailing view being that reducing BACE1 levels will be beneficial in AD, there remains a need to better understand the physiological roles of BACE1 to avoid potential side effects of BACE1 inhibition.

Herein is presented data showing that, in agreement with the previous literature, BACE1 is fundamentally regulated by cell stress. Notably, both acute and prolonged bouts of oxidative and metabolic stress result in significant increases in BACE1 and

APP protein expression. These changes also result in a shift in APP metabolism, with amyloidogenic processing of APP predominating during times of stress.

It has also been shown that chronic elevation of BACE1 and/or manipulation of APP processing can alter cellular glucose uptake and use. These changes were determined through the use of radiolabelled substrate uptake and oxidation as well as extracellular flux assays. These data highlighted a fundamental shift in cellular metabolism, with aerobic glycolysis being utilised over oxidative metabolism of glucose. These changes were later shown to come as a result of metabolic lesions, which acted to impair substrate delivery to the electron transport chain of the mitochondria. Taken together, these data show that overexpression of the AD-associated protein BACE1 phenocopies a number of the earliest detectable changes observed in the brains of people who later develop AD.

Finally, these data highlighted the potential importance of a number of novel pathways (Sirtuin, AMP-activated protein kinase, and peroxisome proliferator-activated receptor-γ coactivator signalling) that may underlie these changes and offer therapeutic avenues for earlier and more targeted treatment to halt AD progression.

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Abbreviations

All abbreviations are defined at first mention in the text. For the convenience of the reader, some of the more commonly used abbreviations are defined below in alphabetical order.

α-KGDH	Alpha-ketoglutarate dehydrogenase
Aβ	β-amyloid
ACC	Acetyl-CoA carboxylase
AD	Alzheimer's disease
ADAM	A disintegrin and metalloproteinase
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
AMPK	AMP-activated protein kinase
APP	Amyloid precursor protein
ATF4	Activating transcription factor 4
BBB	Blood brain barrier
CD38	Cluster of differentiation 38
CMR	Cerebral metabolic rate
CoA	Coenzyme A
DAPT	N-[(3,5-Difluorophenyl)acetyl]-L-alanyl-2-phenyl]glycine-1,1-dimethylethyl ester
DCA	Dichloroacetate
2-DG	2-Deoxy-D-glucose
DNP	Dinitrophenol
ECAR	Extracellular acidification rate
eIF2α	Eukaryotic initiation factor 2α
ETC	Electron transport chain
FCCP	Carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone
fMRI	Functional magnetic resonance imaging
GCN2	General non-derepressible kinase 2
GLUT	Glucose transporter
HFD	High fat diet
HIF1α	Hypoxia-inducible factor 1α
HK	Hexokinase
4-HNE	4-Hydroxynonenal
H_2O_2	Hydrogen peroxide
IDH	Isocitrate dehydrogenase
ISR	Integrated stress response
JNK/SAPK	c-Jun N-terminal kinase/Stress activated protein kinases
MAPK	Mitogen-activated protein kinases
NAD	Nicotinamide adenine dinucleotide
3-NP	3-Nitropropionic acid
OCR	Oxygen consumption rate
PARP1	Poly ADP-ribose polymerase 1
PDH	Pyruvate dehydrogenase
PDK	Pyruvate dehydrogenase kinase
PERK	PKR-like ER kinase
PET	Positron emission tomography
PFK	Phosphofructokinase

PI_3K	Phosphoinositide 3-kinase
PKB	Protein kinase B
PKR	Protein kinase R
PPP	Pentose phosphate pathway
RH	Recurrent hypoglycemia
sAPPα/β	Soluble amyloid precursor protein α/β
SEM	Standard error of the mean
SIRT	Sirtuin
TAPI-1	N-(R)-[2-(Hydroxyaminocarbonyl)methyl]-4-methylpentanoyl-L-naphthylalanyl-L-alanine
TCA cycle	Tricarboxylic acid cycle
uORK	Upstream open reading frames
UTR	Untranslated region