The investigation of the hBCAT proteins in control and
diseased human brains: Implications for glutamate toxicity
in Alzheimer’s disease

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The investigation of the hBCAT proteins in control and diseased human brains: Implications for glutamate toxicity in Alzheimer’s disease

Abstract

Introduction & Aims: The distribution of the BCAT proteins has been extensively mapped in rodent models, and metabolic studies have established that BCAT transamination in the rodent brain is responsible for 30% of de novo glutamate synthesis. However, to date the BCAT proteins have not been mapped to the human brain and their role in pathogenic conditions where glutamate toxicity features has not been investigated. To this end, this study aimed to map the hBCAT proteins to several brain regions. Furthermore, the expression of hBCAT in AD relative to matched controls was investigated and correlated with both physiological and pathological features of AD. Finally, metabolic and inflammatory stimuli were examined for their effect on neuronal expression of hBCATc.

Methods: Distribution of the hBCAT proteins were assessed utilising immunohistochemistry and imaged utilising a 12-bit camera mounted on a Leica DM microscope. Western blot analysis and microscopy determined the expressional difference in AD compared to age and gender matched controls in addition to cell types responsible for the increased expression. Further investigation of neuronal hBCATc expression was examined in the immortal cell line IMR32 utilising Western blot analysis, phase contrast microscopy, flow cytometry and 14C radiolabelled activity assay.

Results & Discussion: For the first time this work demonstrates key differences between the animal model of BCAA metabolism and humans. All brain regions contained cell types labelled for hBCATc and hBCATm. However, while this work mirrored animal models in that hBCATc was localised specifically to neurons, hBCATm was absent from astrocytes and instead labelled the vasculature – contrary to animal models. Another novel finding of this work links altered aminotransferase expression to AD pathology. An increase of hBCATm expression of +117% (p = 2.29 x 10^{-4}) and +143% (p = 7.70 x 10^{-5}) in the frontal and temporal cortex of AD subjects relative to matched controls demonstrates the disease association of hBCATm. A non-significant increase of 32% was observed for hBCATc in the frontal region. With hBCATm expression correlating with Braak stage in both the frontal (p = 1.2 x 10^{-5}, ρ +0.468) and temporal (p = 3.4 x 10^{-4}, ρ+0.391) cortex this work posits that altered BCAA metabolism is occurring simultaneously with AD progression and may be a novel therapeutic target for the treatment of dementia. Another novel aspect of this work also demonstrates cell surface expression of hBCATc and relates this to mTOR signalling. Altered cell surface and protein expression was investigated with functional activity. Together this data demonstrated expressional, functional or activity changes in hBCATc due to glutamate, insulin, leucine, TNFα and IL1α.
Posters, presentations and publications

Posters and presentations

The role of hBCAT in glutamate toxicity, PGR presentation, University of Bristol (2011.04.18)
Expressional alteration of the BCAT enzymes in the AD brain, poster presentation, UWE (2012.01.13)
The role of hBCAT in glutamate toxicity, PGR presentation, UWE (2012.08.09)
Pilot study: Expressional changes of hBCATc in the IMR-32 cell line, implications for neurological disease, poster presentation, UWE (2012.12.19)
Co-localisation of hBCATm protein with LC3-II using confocal and electron microscopy: relation to AD pathology, poster presentation, ARUK conference (2013.02.26)

Papers

Distribution of the branched chain aminotransferase proteins in the human brain and their role in glutamate regulation, published paper (2012)
Upregulation of the BCAT protein in the brains of patients with Alzheimer’s disease: implications in glutamate toxicity (in review)
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Abbreviations

αKG – α-Ketoglutarate
AD – Alzheimer’s disease
ADP – Adenosine diphosphate
ALS – Amyotrophic lateral sclerosis
APS – Ammonium persulphate
ATP – Adenosine triphosphate
BBB – Blood brain barrier
BCAA – Branched chain amino acids
BCKA – Branched chain α-keto acids
BCKD – Mitochondrial branched chain α-keto acid dehydrogenase enzyme
Bim – Bcl-2 interacting mediator of cell death
BOD – Bcl-2 related ovarian death gene
BSA – Bovine serum albumin
BV – Blood vessels
CHAPS – 3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonate
CSF – Cerebrospinal fluid
Cys – Cysteine
DAB – 3,3’-Diaminobenzidine
DAPI – 4’,6-diamidino-2-phenylindole
DIOC6 – 3,3’-dihexyloxacarbocyanine iodide
DTT – Dithiothreitol
EAAT – Excitatory amino acid transporter
EBM – Eagles basal media
Abbreviations

EGM – Endothelial cell growth media
EDTA – Ethylenediaminetetraacetic acid
EGTA – Ethyleneglycoltetraacetic acid
ER – Endoplasmic reticulum
GABA – Gamma-aminobutyric acid
GAPDH – Glyceraldehyde 3-phosphate dehydrogenase
GDH – Glutamate dehydrogenase 1
GLUT – glucose transporter
Grx – Glutaredoxin
GSNO – S-nitrosoglutathione
GSH – Glutathione reduced
GSSG – Glutathione oxidized
hBCATc – Human branched chain aminotransferase (cytosolic isoform)
hBCATm – Human branched chain aminotransferase (mitochondrial isoform)
HEPES – 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HRP – Horseradish peroxidase
IMR32 – Human neuroblastoma cell line
IL – Interleukin
IPTG – Isopropyl β-D-1-thiogalactopyranoside
KIC – Ketoisocaproate
KIV – Keto-isovaleric acid
KMV - Keto-β-methylvalerate
L1 – Large neutral amino acid transporter 1
LDS – Lithium dodecyl sulphate
Abbreviations

nAChR – Nicotinic acetylcholine receptor
NADH – Nicotinamide adenine dinucleotide
NEAA – Non essential amino acids
NMDA – N-methyl-D-aspartic acid
NOS – Nitric oxide synthetase
MMSE – Mini-mental state examination
MSUD – Maple syrup urine disease
mTOR – Mammalian target of Rapamycin
mTORC1 – Mammalian target of Rapamycin complex 1
mTORC2 – Mammalian target of Rapamycin complex 2
NADPH – Nicotinamide adenine dinucleotide phosphate
NO – Nitric oxide
PDI – Protein Disulphide isomerase
PKC – Protein kinase C
PLP – Pyridoxal phosphate
PMP – Pyridoxine monophosphate
PMSF – Phenylmethyl sulfonyl fluoride
RIPA – Radioimmunoprecipitation assay buffer
RPMI – Roswell park memorial institute medium
SDS – Sodium dodecyl sulphate
TBST – Tris-Buffered Saline/Tween
TCA – Trichloroacetic acid
TEMED – Tetramethylethylenediamine
Trx – Thioredoxin
Abbreviations

TNF – Tissue necrosis factor
UO – ubiquionone oxidoreductase
ZIP – zipper interacting protein