Proteomic analysis of the response of cultured neurons to chemical excitation

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Abstract

Mammalian cells secrete a large number of proteins into the extracellular space, the composition and levels of which change as result of environmental factors. However, the identity of most of these secreted proteins has yet to be determined. Recent advances in proteomic technology now allow the isolation and identification of these secreted proteins. Neuronal cell death and injury as a result of excessive and prolonged activation of excitatory amino acid receptors has been termed excitotoxicity, and has been implicated in mechanisms leading to a variety of disease states, such as Alzheimer's disease. Available evidence suggests that under excitotoxic stress, neurons secrete signals that can alter the function of neurons with which they are connected via synapses or to which they are proximate. Many of these signals act via volume transfer, diffusing via the ECF to act on populations of cells, to induce slow long-lasting effects. The successful isolation and *in vitro* culture of rat cortical cells is reported here, These cultures are 95 % pure for neurons, as determined by imunocytochemistry. They are viable for more than 14 days under B27-supplemented culturing conditions, and are viable for up to a further 3 days under protein-free culturing conditions. Live dead/assays, utilising fluorescein diacetate and propidium iodide, demonstrated that cell survival remained high even after 72 h exposure to 50 μM kainic acid. Analysis of conditioned medium using 2D-PAGE combined with MALDI-TOF MS demonstrated significant proteolysis of the bovine serum albumin protein. This proteolysis only occurred in the presence of the cortical cells. In addition, immunocytochemistry confirmed that both neurons and glia had taken up the bovine serum albumin. Increasing evidence implicates cerebrovascular dysfunction, in particular the breakdown of the blood-brain barrier, as an early event in the development of Alzheimer's disease. Elevated intracellular Cu^{2+} levels are also seen in the Alzheimer's disease brain, and are thought to be responsible for neuronal cell death through metal-mediated oxidative stress. It is proposed here that the albumin molecule, which binds $Cu^{2+}$with high-affinity, gains entry to the CNS as a result of BBB dysfunction. Neuronal uptake and processing of the albumin molecule, as demonstrated here, resulting in the intracellular release of Cu^{2+}, may be the mechanism by which the elevated Cu^{2+} levels seen in Alzheimer's disease occur. In addition, initial analysis of the conditioned medium of cortical neurons stimulated with 50 μM KA for 72 h, suggests that processing of the bovine serum albumin is altered, with some fragments up-regulated and others down-regulated.

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Abbreviations and chemical formulae

A	
~	approximately
A	ampere
aa	amino acid
Aβ	β-amyloid
Ab	antibody
ACh	acetylcholine
αCHC	alpha-cyano-4-hydroxycinnamic acid
AD	Alzheimer's disease
Ag	antigen
AMPA	alpha-amino-3-hydroxy-5-methylsoxazole-4-propionate
AOP2	antioxidant protein 2
AP	action potential
B	
BBB	blood-brain barrier
bFGF	basic fibroblast growth factor
BPB	bromophenol blue
BSA	bovine serum albumin
C	
°C	degrees Celsius
Ca^{2+}	calcium ion
cDNA	complementary DNA
CNS	central nervous system
CSF	cerebrospinal fluid
Cu^{2+}	copper (II) ion
D	
Da	Dalton
DMEM	Dulbecco's Modified Eagle's Medium
DTT	dithiothreitol
E	
ECF	extracellular fluid
F	
FCS	fetal calf serum
FD	fluorescein diacetate
FGF	fibroblast growth factor
FITC	fluorescein isothiocyanate
G	
x g	relative centrifugal force
g	gram/s
GABA	gamma-amino butyric acid
GFAP	glial fibrillary acidic protein
GH	growth hormone
GluR	glutamate receptor
H	
h	hour/s
H_2O_2	hydrogen peroxide
H_2O	water
HB-A	Hibernate-A medium

	HCl	hydrochloric acid
	HRP	horseradish peroxidase
	HSA	human serum albumin
I		
	IAA	iodoacetamide
	IEF	isoelectric focusing
	IgG	immunoglobulin G
	iGluR	ionotropic glutamate receptor
	IPG	immobilised pH gradient
K		
	k	kilo
	KA	kainic acid
L		
	L	litre
	LDCV	large dense core vesicles
	LDH	lactate dehydrogenase
M		
	-m	metre or milli
	M	molar
	MALDI	matrix assisted laser desorption ionisation
	MCAO	middle cerebral artery occlusion
	MeCN	acetonitrile
	-µ	micro (1×10^{-6})
	min	minute/s
	mRNA	messenger RNA
	MS	mass spectrometry
	MW	relative molecular weight
	MWCO	molecular weight cut-off
	m/z	mass to charge ratio
N		
	#	number
	NaCl	sodium chloride
	-n	nano (1×10^{-9})
	NaOH	sodium hydroxide
	NB-A	Neurobasal-A medium
	NBF	neutral buffered formalin
	NCBI	National Centre for Biotechnology Information
	NeuN	neuronal nuclei
	NGF	nerve growth factor
	NH_4HCO_3	ammonium bicarbonate
	NMDA	N-methyl-D-aspartate
	NT	neurotransmitter
O		
	$O_2^{\bullet}$	superoxide
	$OH^{\bullet}$	hydroxyl radical
P		
	PAGE	polyacrylamide gel electrophoresis
	PARP	poly (ADP ribose) polymerase
	PBS	phosphate buffered saline
	PFA	paraformaldeyhyde
	pH	power of hydrogen

	pI	isoelectric point
	PI	propidium iodide
	ppm	parts per million
R		
	rpm	revolutions per minute
	RT	room temperature (21°C)
S		
	5HT	serotonin
	SDS	sodium dodecyl sulphate
	SV	small vesicle
T		
	2D	two-dimensional
	TFA	trifluoroacetic acid
	TOF	time of flight
	TTBS	tris-buffered saline with Tween-20
	2D-PAGE	two-dimensional polyacrylamide gel electrophoresis
U		
	UoA	University of Auckland
	US	unstimulated
	UV	ultraviolet
V		
	V	voltage
	VT	volume transmission
	v/v	volume per volume
W		
	WT	wiring transmission
	w/v	weight per volume
	w/w	weight per weight

Company abbreviations

Adobe	Adobe California, USA
Amersham Biosciences	Amersham Biosciences Uppsala, Sweden
Applichem	AppliChem GmbH Darmstadt, Germany
Applied Biosystems	Applied Biosystems California, USA
Axis-Shield	Axis-Shield Group Oslo, Norway
BDH	BDH Laboratory Supplies Poole Dorset, UK
Bio-Rad	Bio-Rad Laboratories California, USA
Brainbits-USA	Brainbits-USA Illinois, USA
Chemicon	CHEMICON International California, USA
Falcon	Falcon Michigan, USA
GraphPad	GraphPad Software Inc. California, USA
Hansen and Rosenthal	Hansen & Rosenthal KG Hamburg, Germany
Invitrogen-GIBCO	Invitrogen Corporation Carlsbad, USA
May and Baker	May and Baker Essex, UK
Medlab	Diagnostic Medlab Auckland, New Zealand
Molecular Probes	Molecular Probes Oregon, USA
Perkin-Elmer	PerkinElmer Life and Analytical Sciences Massachusetts, USA
Promega	Promega Wisconsin, USA
Sartorius	Sartorius AG Goettingen, Germany
Scharlau	Scharlau Chemie Barcelona, Spain
Serva	SERVA Electrophoresis GmbH Heidelberg, Germany
Sigma	Sigma-Aldrich Missouri, USA
Zymed	Zymed Laboratories, Inc. California, USA