THE ROLE OF THE INDUCIBLE TRANSCRIPTION FACTORS IN STATUS EPILEPTICUS-INDUCED DELAYED NEURONAL DEATH.

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ABSTRACT.

Status epilepticus (SE) is a serious neurological disorder, characterised by prolonged and/or frequent seizure activity. Following SE, a selective and delayed neuronal death (DND) occurs in limbic regions of the brain, particularly in the hippocampus. The objective of this thesis was to investigate the molecular basis of SE-induced DND in the Wistar rat hippocampus.

Following the induction of SE, moribund (i.e. dead/dying) neurons were identified by histological staining, DNA fragmentation and an increase in activated microglia. Clusterin, a glycoprotein implicated in apoptotic cell death was also observed to accumulate in the soma and axons of moribund neurons 72-144 hr following SE. Morphological evidence suggested that dying neurons exhibited many of the classical features of apoptosis (i.e. apoptotic body formation, oligo-nucleosomal DNA fragmentation and rapid phagocytosis of debris) and therefore raised the possibility that SE-induced DND might be programmed (i.e. requiring *de novo* protein synthesis).

To investigate this hypothesis I have examined the temporal and anatomical expression of a number of proteins which may have a critical role in SE-induced DND. The expression of the inducible transcription factors (ITFs) was examined as they couple extracellular stimulation to the transcription of late effector gene(s), resulting in long-term phenotypical changes in the neuron and therefore, they may couple SE-inducing stimulation with DND. A high correlation was shown between neurons which exhibited a delayed and prolonged ITFP expression and those which were selectively vulnerable to SE-induced DND (e.g. CA1 and CA3 pyramidal cells and dentate hilar neurons). However, administration of the protein synthesis inhibitor anisomycin following the induction of SE reduced the ITFPs expression, but resulted in an increase in SE-induced DND after 48 hr. However, the levels of brain-derived neurotrophic factor (BDNF)-like immunoreactivity were also shown to attenuate at this time after this procedure. Thus, protein synthesis inhibitors administered following SE may attenuate the level of trophic support and promote cell death.

To further investigate the role of the ITFPs in nerve cell death, etoposide, a DNA topoisomerase II inhibitor, which is known to facilitate apoptosis was infused into the hippocampus. The results suggested that a complex ITFP expression occurred which preceded nerve cell death. Moreover, this nerve cell death occurred earlier (12-24 hr) and was not anatomically selective. Furthermore, following the etoposide infusion, clusterin was expressed in the hippocampal pyramidal cells, in the dentate hilar neurons and in the dentate granule cells, however the latter exhibited the strongest BDNF-like immunoreactivity.

In summary, circumstantial evidence suggests that the ITFPs may form a critical component in the cascade of events which couple toxic stimulation to nerve cell death. However, this thesis demonstrates that the ITFPs have a complex role in DND, as although the ITFPs may be sufficient to induce DND, they may not always be necessary (e.g. in the absence of sufficient trophic support).

PUBLICATIONS RESULTING IN PART OR FULL FROM THIS THESIS.

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TABLE OF CONTENTS

LIST OF FIGURES.

LIST OF TABLES.

ABBREVIATIONS.

^{35}SdATP	^{35}S-deoxyadenosine 5′-[α-thio]triphosphate
AED	anti-epileptic drug
AHS	Ammon's horn sclerosis
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid
ANI	anisomycin (2-[p-methoxybenzyl]-3,4-pyrrolidinediol 3-acetate)
ANOVA	analysis of variance
AP	anterior posterior
AP-1	activator protein-1
ATP	adenosine triphosphate
β-AP	β-amyloid protein
β-APP	β-amyloid precursor protein
BDNF	brain-derived neurotrophic factor
bFGF	basic fibroblast growth factor
bp	base-pair
BSA	bovine serum albumin
CA1-3	Cornu Ammonis 1-3
Ca^{2+}	calcium ion
CaBP	calcium binding proteins
cAMP	cyclic adenosine monophosphate
Cdk	cyclin dependent kinases
cDNA	complementary DNA
CHS	continuous hippocampal stimulation
CHS/ANI	continuous hippocampal stimulation followed by an anisomycin infusion (icv)
CHS/VEH	continuous hippocampal stimulation followed by a vehicle infusion (icv)
CHX	cycloheximide
CNS	central nervous system
DAB	3,3′-diaminobenzidine.4 hydrochloric acid
DAG	1,2-diacylglycerol

dH_2O	distilled water
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DND	delayed neuronal death
DPM	disintergrations per minute
DTT	dithiothreitol
EDTA	ethylene diaminetetra acetate
EEG	electroencephalograph
FP-1	fusion protein-1
FP-2	fusion protein-2
G0, G1, G2	gap 0, 1, 2 phase (of cell cycle)
GABA	γ-aminobutyric acid
GAP 43	growth associated protein-43
H_2O_2	hydrogen peroxide
HI	hypoxic-ischemia
hr	hour
HSP	heat-shock protein
IB4	isolectin-B4
ICE	interleukin-1β-converting enzyme
i.c.v.	intracerebroventricular
IGF	insulin-like growth factors
$Ins(1,4,5)P_3$	inositol-1,4,5-triphosphate
ITF	inducible transcription factor
ITFP	inducible transcription factor protein
ip	intraperitoneal
kb	kilobase
kg	kilograms
L	lateral
LEG	late effector gene
LEGP	late effector gene protein
mL	milli-litres
M	mitosis phase (of cell cycle)
MAP kinase	mitogen-activated protein kinase

min	minute
MK-801	dizocilipine maleate or (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptene-5,1-imine maleate
mRNA	messenger ribonucleic acid
NGF	nerve growth factor
NMDA	*N*-methyl-*D*-aspartate
NT	neurotrophin
PB	phosphate buffer
PBS	phosphate buffered saline
PC12	pheochromocytoma cell line
PCD	programmed cell death
PCP	phencyclidine
PFA	paraformaldehyde
PKA	protein kinase A
PKC	protein kinase C
PMSF	phenylmethylsulfonyl fluoride
PNS	peripheral nervous system
Rb	retinoblastoma gene
RNAase	ribonuclease
S	DNA synthesis phase (of cell cycle)
SAPK	stress activated protein kinase
SE	status epilepticus
SGP-2	sulphated glycoprotein-2
SOD	super oxide dismutase
SSC	standard saline-citrate
TAE	tris-acetate/EDTA buffer
TBS	tris buffered saline
TBST	tris bufferfed saline containing 0.05% (v/v) Tween-20
TdT	terminal deoxynucleotidyl transferase
TGF	transforming growth factor
TLE	temporal lobe epilepsy
TNF	tumour necrosis factor
trk	tyrosine-kinase-linked neurotrophic receptor

TUNEL(+)	TdT-mediated dUTP-biotin nick end labelling positve staining
V	ventral
v/v	volume/volume
VSCC	voltage-sensitive calcium channel
w/v	weight/volume