Characterisation of ERK Distribution and Activity in Rat Pheochromocytoma Cells

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ABSTRACT

Nerve growth factor (NGF) binds to the NGF receptor, TrkA, at the tips of nerve cell axons, sending a signal that prevents programmed cell death and causes survival, growth, and differentiation of the nerve cell. Both NGF and TrkA have been demonstrated to be retrogradely transported from axon tips to nerve cell bodies, however the mechanism of this transport, and its function, is strongly debated. Using a recently developed cell fractionation protocol in conjunction with *in vitro* reactions using an ATP regenerating system, our lab has isolated small vesicles containing NGF bound to activated TrkA. These vesicles may provide a vehicle for retrograde transport of the NGF signal and initiation of signal transduction in the cell body.

ERK1 is a serine/threonine kinase that is activated by NGF-activated TrkA. Prolonged ERK1 activity is characteristic of cells stimulated by NGF. The purpose of the experiments in this thesis was to characterise the intracellular distribution and activity of ERK1 before and after NGF stimulation, in rat pheochromocytoma (PC12) cells, which are a good model for nerve cells. We have found that ERK1 activity is redistributed between cell compartments after NGF stimulation of PC12 cells. ERK1 activity increased in sedimentable fractions that emerged from mechanically permeabilised cells after NGF treatment and *in vitro* reactions with ATP. Importantly, the results from glycerol velocity gradient experiments showed that ERK1 was not associated with membranes. Instead ERK1 was found in a rapidly sedimenting particle whose sedimentation was not affected by detergent solubilisation. These results suggest that ERK1 is recruited into a protein complex, after activation, which may be an important step in signal transduction. Formation of this complex is likely to be downstream of signalling vesicles containing NGF bound TrkA.

LIST OF ABBREVIATIONS

AMP	Adenosine monophosphate
APS	Ammonium persulphate
ATP	Adenosine triphosphate
BB	Bud buffer
BDNF	Brain-Derived Neurotrophic Factor
BSA	Bovine serum albumin
ChAT	Choline acetyltransferase
CNS	Central nervous system
COS	Transformed African Green Monkey kidney cell line
CRE	Cyclic AMP response element
CREB	Cyclic AMP response element binding protein
ddH_2O	Double-distilled water
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
DTT	Dithiothreitol
ECL	Enhanced chemi-luminescence
EDTA	Ethylenediamine tetraacetic acid
EGFR	Epidermal growth factor receptor
EGTA	Ethylene glycol-bis(β-aminoethyl ether) N,N,N',N'-tetraacetic acid
ERK	Extracellular regulated kinase
FGF	Fibroblast growth factor
GAP	GTPase activating protein
GDP	Guanosine diphosphate
GST	Glutathione S-transferase
GTP	Guanosine triphosphate
Grb2	Growth factor receptor binding protein 2
IEG	Immediate early gene
IP.	Immunoprecipitation
kDa	Kilo Dalton
LRG	Late response gene
MAPK	Mitogen activated protein kinase

MEK	MAPK or ERK Kinase
MOPS	3-(N-Morpholino)propanesulphonic acid
mRNA	messenger ribonucleic acid
m-Sos	Mammalian Son-of-Sevenless
NGF	Nerve growth factor
NIH 3T3	Contact inhibited NIH Swiss mouse embryo cell line
NT	Neurotrophin
$p75^{NTR}$	p75 neurotrophin receptor
PBS	Phosphate buffered saline
PC12	Adrenal Pheochromocytoma rat cell line
PCD	Programmed cell death
PEE	PBS with EDTA and EGTA
PGB	PBS with glucose and BSA
PI-3 kinase	Phosphatidylinositol-3' kinase
PKC	Protein kinase C
PLC-γ1	Phospholipase C gamma 1
PMSF	Phenylmethylsulphonyl fluoride
PNS	Peripheral nervous system
PtdIns	Phosphatidylinositol
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SH	Src homology
SRF	Serum response factor
SY5Y	SK-N-SH-SY5Y human neuroblastoma cell line
TCA	Trichloroacetic acid
TBS	Tris buffer saline
TEMED	N,N,N',N' tetramethyl ethylene diamine
TH	Tyrosine hydroxylase
Tris	Tris(hydroxymethyl)aminoethane
Triton X-100	t-Octylphenoxypolyethoxyethanol
Trk	Tyrosine receptor kinase
Tween 20	Polyoxyethylenesorbitan monolaurate
Tween 40	Polyoxyethylenesorbitan monopalmitate
v/v	Volume/volume
w/v	Weight/volume

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