IMIDAZOLINE RECEPTOR ANTISERA-SELECTED PROTEIN: A UNIQUE MODULATOR OF NEURONAL DIFFERENTIATION

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Table of Contents

List of Tables

List of Figures

Abstract

The imidazoline I_1 receptor (I_1-R) is a novel receptor found primarily in the brain and nervous tissue where it modulates neurotransmission. It is named for its high affinity for compounds with an imidazoline structure such as the anti-hypertensive drugs, clonidine and moxonidine.

The imidazoline receptor antisera-selected protein (IRAS) is the putative clone of the I_1-R. IRAS has a unique structure, which does not resemble any other receptor protein. IRAS is present throughout the body with highest levels in the brain. There is a growing body of research examining the physiological roles of IRAS as an I_1-R, in cell survival, migration and protein trafficking. However, there is little research into its neuronal functions.

IRAS interacts with other membrane receptors: the mouse homologue of IRAS reorganises the actin cytoskeleton through interaction with the $\alpha5\beta1$ fibronectin receptor. IRAS also binds insulin receptor substrate 4 and enhances insulin-induced extracellular signal-regulated kinase1/2 (ERK1/2) activation. Actin reorganisation and ERK1/2 activation are important for the development of neurites during neuronal differentiation. Therefore, the work described in this thesis aimed to investigate the effects of IRAS on neuronal differentiation. Studies reported in this thesis also aimed to investigate whether IRAS affected ERK1/2 signalling of other receptors involved in neuronal differentiation such as the NGF receptor, TrkA, and lysophospholipid receptors.

The above aims were carried out in neuronal model PC12 cells transfected with either IRAS or a vector plasmid. Fluorescence microscopy and Western blotting techniques were used to examine the effect of IRAS on cell morphology and ERK1/2 signalling.

The work described in this thesis found that IRAS reorganises the actin cytoskeleton and enhances growth cone development in PC12 cells. This study also shows that IRAS differentially enhances or inhibits NGF-induced PC12 cell differentiation depending on the presence or absence of serum in the media. In full-serum conditions, IRAS enhanced neurite outgrowth and this was accompanied by an increase in ERK1/2 activation. In serum-starved cells, IRAS inhibited neurite outgrowth with similar levels of ERK1/2 activation observed in vector- and IRAS-transfected cells. Finally, studies presented in this thesis found that IRAS enhances lysophosphatidic acid-induced ERK1/2 activation and that IRAS interacting with lysophospholipid receptor agonists present in serum is a potential mechanism by which it enhances NGF-induced ERK1/2 activation in full-serum conditions.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Signed:…… Date:…….

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Abbreviations

Alzheimer’s disease	AD
Central nervous system	CNS
Diacylglycerol	DAG
Epidermal growth factor	EGF
Extracellular signal-regulated kinase	ERK
G-protein coupled receptor	GPCR
I_1 Receptor	I_1-R
Imidazoline antisera-selected	IRAS
Lysophosphatidic acid	LPA
Lysophospholipid	LP
Mitogen activated protein	MAP
μ opioid receptor	MOR
Nerve growth factor	NGF
Phosphatidylcholine-specific phospholipase C	PC-PLC
Phosphatidylinositol-3-phosphate	PI3P
Phosphoinositide	PI
Protein kinase C	PKC
Protein tyrosine kinase	PTK
Rat embryonic fibroblast	REF
Rostral ventrolateral medulla	RVLM
Sorting Nexin	SNX
Sphingosine-1-phosphate	S1P
Sphingosine kinase	SphK