The mechanism of glucose-induced RCAN1 expression in β-cells

Rakhvinder Kaur Kashmir Singh
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Supervisor: Dr. Damien Keating

Centre for Neuroscience,

Department of Human Physiology,

Flinders University

School of Medicine,

Adelaide, South Australia.

Dedication

This thesis is dedicated to my mother

Harcharan Kaur Dhillon

(1937-2011)

This work contains no material which has been accepted for the award of any other degree or diploma in any university of other tertiary institution and, to the best of my knowledge and belief, the thesis 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.

Rakhvinder Kaur Kashmir Singh

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ABSTRACT

Regulator of calcineurin 1 (RCAN1) is a gene located on chromosome 21 that is highly expressed in the brain, heart, and also in numerous endocrine cells. RCAN1 is an endogenous inhibitor of the protein phosphatase calcineurin which is essential for β cell function and circulatory insulin levels. Changes in RCAN1 expression regulate exocytosis, mitochondrial function, apoptosis, proliferation and susceptibility to oxidative stress. Previous work with RCAN1 in β cells, showed that elevated expression of RCAN1 has a profound effect on β cell function resulting in altered gene expression, elevated ROS accumulation, reduced insulin secretion, hypoinsulinemia and fasting hyperglycemia. Furthermore, an increase in RCAN1 expression is also seen in chronic hyperglycemia in pancreatic islets. However, the mechanisms underlying this in β cells are unknown. As RCAN1 expression is induced by oxidative stress in neuronal cultures and oxidative stress can occur in β cells during periods of cell stress such as hypoxia, there could potentially be a role of RCAN1 in hypoxia. Hence, the aims of this study were firstly to investigate potential mechanisms underlying the glucose-induced expression of RCAN1 followed by the effects of hypoxia on the expression of RCAN1 in MIN6 β cells.

Glucose induction of both the RCAN1 isoforms, RCAN1-1 and RCAN1-4, was dependent on the production of ROS and Ca^{2+} entry in the MIN6 β cells at the gene and protein level. This is because reversal of the glucose-induction of these RCAN1 isoforms was seen in the presence of antioxidants and Ca^{2+} channel blockers. However, the inhibition of calcineurin via the usage of FK506 and Cyclosporine A was seen to effect the expression of RCAN1-4 significantly at

the gene and protein level but not that of RCAN1-1. These results suggest a common ROS and Ca^{2+}-associated pathway for the control of both RCAN1 isoforms but a calcineurin-associated pathway regulating RCAN1-4 expression but not RCAN1-1. Hypoxia at various levels and durations had no effect on the expression of either of the RCAN1 isoforms. This study provides new insights into the mechanisms by which glucose induces the expression of RCAN1. Given that increased RCAN1 has negative effects on β cell function and induces diabetes, the findings further our understanding of the mechanisms linking chronic high glucose and β cell dysfunction.

Commonly Used Abbreviations

µM	Micromole
ADP	Adenosine diphosphate
ANT	Adenine nucleotide translocator
AP-1	Activator protein-1
Arx	Aristaless related homeobox
ATP	Adenosine triphosphate
Bcl-2	B-cell lymphoma-2
bp	Base pairs
Cabin1	Calcineurin binding protein 1
CDK4	Cyclin-dependent kinase 4
CoQ	Coenzyme Q
CPT-1	Carnitine-palmitoyl transferanse-1
CsA	Cyclosporine A
DAPI	4’,6-diamino-2-phenylindole,dihydrochloride
DNA	Deoxyribose nucleic acid
DMEM	Dulbecco’s Minimal Essential Medium
DMSO	Dimethyl sulphoxide

ELISA	Enzyme-linked immunosorbent assay
FAD	Flavin adenine dinucleotide
FFA’s	Free fatty acids
FKBP	FK-binding proteins
FLIP	FLICE inhibitory protein
GAD	Glutamic acid carboxylase
GLUT1	Glucose transporter 1
GLUT2	Glucose transporter 2
GLUT4	Glucose transporter type 4
GSK-3β	Glycogen synthase kinase 3β
GTP	Guanosine triphosphate
Hes-1	*Hairy and Enhancer* of split homologue-1
HLA	Human leucocyte antigen
HNF	Hepatocyte nuclear factor
IA-2	Islet specific antigen-2
IL	Interleukin
Insm1	Insulinoma-associated 1
IRS	Insulin receptor substrate
K_{ATP}	ATP sensitive potassium channels

K_m	Michaelis constant
kDa	Kilo Dalton
LC-CoA	Long-chain acyl-CoA esters
MaFA	Musculoaponeurotic fibrosarcoma oncogene homolog A
MIN6	Mouse Insulinoma
mg	Milligram
ml	Millilitre
mM	Millimolar
MODY	Maturity-onset diabetes of the young
mRNA	Messenger ribonucleic acid
NAD	Nicotinamide adenine dinucleotide
NEFA	Non-esterified fatty acids
Neurod-1	Neurogenic differentiation-1
NFAT	Nuclear factor of activated T-cells
Ng3	Neurogenin 3
NHR	NFAT homology region
NLS	Nuclear localisation sequence
nm	Nanometre
PAX4	Paired box gene 4

PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
Pdx-1	Pancreatic and duodenum homeobox 1
PKC	Protein-kinase C
PP1	Protein phosphtase-1
PP2	Protein phosphtase-2
PPARγ	Peroxisome proliferator-activated receptor gamma
PPI	Peptidyl-propyl cis-trans isomerase
Ptf1a	Pancreas transcription factor 1 alpha
RCAN1	Regulator of calcineurin 1
RCANs	Regulators of calcineurin
ROS	Reactive oxygen species
RRP	Readily releasable pool
RT-PCR	Reverse transcription polymerase chain reaction
SIRT1	sirtuin – (silent-mating type information regulation 2- homolog)-1)
SNAP-25	Synaptosomal-associated protein 25
SNARE	Soluble NSF attachment protein receptor
TBE	Tris boric acid EDTA buffer
TBS	Tris buffered saline

TCA	Tricarboxylic acid
VDCC	Voltage dependent Calcium channels