Effect of Adrenomedullin on Insulin Resistance in Type 2 Diabetic Rats

Thesis

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ABSTRACT

Non-insulin-dependent diabetes mellitus is one of the common metabolic disorders that ultimately afflict the majority of individuals. Adrenomedullin (AM) is a potent vasodilating peptide originally isolated from human pheochromocytoma and it was found latter to be produced by many tissues. Previous studies reported development of insulin resistance in aged Am deficient mice. In this study, a single tail vein gene delivery approach was employed to explore its potential role in insulin resistance. Upregulating AM gene in type 2 diabetic rats (high fat diet+ streptozotocin injection) significantly improved skeletal muscle glucose uptake, serum glucose, insulin, cholesterol and triglycerides as well as the insulin resistance index (HOMA-IR) compared with diabetic rats not receiving AM gene. The beneficial effects of AM gene delivery were accompanied by increased muscle GLUT-4 gene expression, indicating that AM is closely related to skeletal muscle insulin responsiveness. These findings provide new insights into the role of AM in insulin resistance and may have significance in therapeutic applications in type 2 diabetes.

KEY WORDS:

Adrenomedullin -Insulin resistance -Type 2 diabetes.

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Amy Fakhry Boushra

Dedicated To

My beloved parents

My brother

&

My friends

For their support and patience

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LIST OF ABBREVIATIONS

ADA	American Diabetic Association
ADP	Adenosine diphosphate
AM	Adrenomedullin
AM2	Intermedin
AM_1 receptor	Adrenomedullin receptor type 1
AM_2 receptor	Adrenomedullin receptor type 2
AMBP-1	Adrenomedullin binding protein-1
ANOVA	Analysis of variance
aP2	Adipocyte P2 gene
AP-2	Activator protein- 2
AT_1	Angiotensin II receptor type 1
ATP	Adenosine triphosphate
bp	Base pair
cAMP	Cyclic adenosine monophosphate
cDNA	Complementary deoxy-ribonucleic acid
CECs	Cerebral endothelial cells
cGMP	Cyclic guanosine monophosphate
CGRP	Calcitonin gene-related peptide
COOH-terminal	Carboxy-terminus
CRLR (CLR)	Calcitonin receptor-like receptor
CSF	Cerebrospinal fluid
CT	Calcitonin
dATP	Deoxy adenosine triphosphate
dCTP	Deoxy cytidine triphosphate
DEPC	Diethyl pyrocarbonate
dGTP	Deoxy guanosine triphosphate
DM	Diabetes mellitus
DNA	Deoxy-ribonucleic acid
DNase 1	Deoxy-ribonuclease 1
dNTPs	Deoxynucleotide triphosphates
DOPE	lipdioleoyl phosphatidylethanolamine
DOSPA	2,3-dioleyloxy-N-[2(sperminecarboxamido)ethyl]-N,N-dimethyl-1-propanaminium trifluoroacetate

dTTP	Deoxy thymidine triphosphate
EB	Ethidium Bromide
EDHF	Endothelium-derived hyperpolarizing factor
EDTA	Ethylene diamine tetraacidic acid
ELISA	Enzyme linked immuno sorbant assay
eNOS	Endothelial nitric oxide synthase
ENPP1	Ectonucleotide pyrophosphatase/phosphodiesterase
FFAs	Free fatty acids
FSH	Follicular stimulating hormone
Gab-1	Growth factor receptor binding protein-2 associated binder-1
GABA	Gamma amino butyric acid
GIP	Glucose-dependent insulinotropic polypeptide
GLP-1	Glucagon like peptide-1
GLUT-4	Glucose transporter-4
G-protiens	Guanosine triphosphate (guanosine nucleotide)-binding proteins
GPCRs	G- protein coupled receptors
Grb-2	Growth factor receptor binding protein-2
Gs	s type of G-proteins
GSK3	Glycogen synthase kinase-3
GTC	Guanidine thiocyanate
GTPases	Guanosine triphosphatases
HbA1c	Glycosylated hemoglobin
HDL	High density lipoprotein
HFD	High fat diet
HIF-1	Hypoxia-inducible factor 1
HOMA-1R	Homeostasis model assessment of insulin resistance
HPRI	Human placental ribonuclease inhibitor
iAM	Intermediate inactive form of adrenomedullin
^{125}I-AM	Radioactive iodine labeled adrenomedullin
^{125}I-CGRP	Radioactive iodine labeled calcitonin gene related peptide
$I_{Ca\text{-}L}$	L-type calcium current (long lasting Ca^{2+} channels)
IgA	Immunoglobulin A
IGF-I	Insulin-like growth factor-I

IL-1ß	Interleukin-1 Beta
IL-6	Interleukin-6
iNOS	Inducible nitric oxide synthase
IR	Insulin receptor
IRS (1-4)	Insulin receptor substrates
K_{ATP}	Adenosine triphosphate-sensitive potassium channels
K_{Ca}	Calcium-activated potassium channels
KDa	Kilo dalton amino acid
KR–IGF-IR	Dominant-negative insulin-like growth factor-I receptor
LADA	Latent autoimmune diabetes of the adult
LDL	low density lipoproteins
LPS	Lipopolysaccharide
LV	Left ventricle
mAM	Mature adrenomedullin
MAPK	Mitogen activated protein kinase
MMLV	Moloney murine leukemia virus
MODY	Maturity onset diabetes of the young
mRNA	Messenger ribonucleic acid
MR-proAM	Midregional proadrenomedullin
NAD	Nicotinamide adenine dinucleotide
NADH	Reduced nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
NF-κB	Nuclear factor-kappa B
NGF	Nerve growth factor
NH2-terminus	Amino-terminus
NO	Nitric oxide
OD	Optical density
P	Probability
PAI-1	Plasminogen activator inhibitor -1
PAMP	Proadrenomedullin N-terminal peptide
PASMCs	Pulmonary artery smooth muscle cells

PC-1	Plasma cell differentiation factor-1
PCR	Polymerase chain reaction
PDGF	Platelet-derived growth factor
PDK 1	Phosphoinositide-dependent kinase 1
PEPCK	Phosphoenolpyruvate carboxykinase
pH	Power of hydrogen
PH	Pleckstrin homology
PI3-K	Phosphatidylinositol 3-kinase
PIP3	Phosphatidylinositol 3, 4, 5- phosphate
PKA	Protein kinase A
PKB/cAkt	Protein kinase B, Akt (protein serine/Threonine kinase)
PKC (α, ß2, λ , ζ, θ, **ε, δ)**	Protein kinase C (alpha, beta2, lambda, zeta, theta,epsilon, delta)
pM	Picomole
PPAR(α, δ, and)γ	Peroxisome proliferator-activated receptor (alpha, delta, gamma)
PreproAM	Preproadrenomedullin (adrenomedullin precursor)
ProAM	Proadrenomedullin
PTP	Protein tyrosine phosphatase
Rad	Ras associated with diabetes
RAECs	Rabbit aortic endothelial cells
RAMPs (1-3)	Receptor activity modifying proteins
RCP	Receptor component protein
RNase	Ribonuclease
ROS	Reactive oxygen species
rpm	revolutions per minute
RT	Reverse transcriptase
SD	Standard deviation
SE	Standard error
STZ	Streptozotocin
TAE	Tris-Acetate EDTA buffer
Taq	Thermus aquaticus
TNF-α	Tumor necrosis factor- alpha
TrkA	Tropomyosin-related kinase A receptors

TZDs	Thiazolidinediones
UCP-2	Uncoupling protein 2
UV	Ultraviolet
VSMCs	Vascular smooth muscle cells
ZG	Zona glomerulosa

Introduction and Aim of the work

Adrenomedullin (AM) is a potent 52-amino acid vasodilator peptide originally isolated from tissue extracts of human pheochromocytoma ***(Kitamura et al, 1993 (A)).*** Moreover it has been detected in a variety of organs, such as adrenal gland, kidney, heart, lung, spleen, and brain, as well as in endothelial and vascular smooth muscle cells ***(Dobrzynski et al, 2000).***

Adrenomedullin is involved in a variety of biological activities, including vasodilation, diuresis, and inhibition of aldosterone secretion. Plasma AM levels are increased in patients with cardiac hypertrophy, heart failure, renal dysfunction, and hyperglycemia acting in an autocrine/paracrine and endocrine fashion ***(Dobrzynski et al, 2002).***

Hypertension and insulin resistance, are common related disorders that are often associated with increased oxidative stress and the resultant vascular damage. AM was commonly identified as vasorelaxant and hypotensive in cardiovascular diseases ***(Brain and Grant, 2004),*** however some studies investigated its role in insulin resistance ***(Shimosawa et al, 2003).***

Adrenomedullin-deficient mice not only showed increased production of muscular reactive oxygen species, but also had significantly decreased insulin-stimulated glucose uptake into the soleus muscle associated with impairment of insulin signals. In turn, these abnormalities could be nearly reversed by either treatment with a membrane-permeable superoxide dismutase mimetic, or adrenomedullin supplementation ***(Finkel and Holbrook, 2000 and Shimosawa et al, 2003).***

The aim of the present study is to investigate the effect of upregulation of adrenomedullin gene expression on insulin resistance in type 2 diabetes.

Adrenomedullin

Structure and Synthesis of Adrenomedullin

Adrenomedullin (AM) was isolated originally by ***Kitamura et al, 1993 (A)*** from a human pheochromocytoma. The characterization of purified human AM showed that it is a 52-amino acid peptide with a single disulfide bridge and an amidated tyrosine at the carboxy terminus. It shows some homology with calcitonin gene-related peptide (CGRP) and has therefore been added to the calcitonin/CGRP/amylin peptide family. Rat adrenomedullin has 50 amino acids, with 2 amino acid deletions and 6 amino acid substitutions compared with the human AM ***(Sakata et al, 1993)*.**

Adrenomedullin is synthesized as part of a larger precursor molecule, termed preproadrenomedullin. In both rat and human this precursor consists of 185 amino acids ***(Kitamura et al, 1993 (B) and Sakata et al, 1993).*** The adrenomedullin precursor gives rise to several biologically active cleavage products. These are: proadrenomedullin N-terminal peptides **(PAMP**) including proadrenomedullin N-terminal peptide of 20 amino acids {PAMP (1-20)} and proadrenomedullin N-terminal peptide of 12 amino acids {PAMP(9-20)}**,** **Adenotensin** {proAM (153-185)}, and **prodepin** {proAM (22-41)} appears to be a rat-specific adrenomedullin cleavage product ***(Kitamura et al, 1993 (B), Lippton et al, 1994 (B) and Gumusel et al, 1995)***. AM2 (intermedin) is a 47-amino-acid novel member of the calcitonin/CGRP family peptides discovered simultaneously by two groups ***(Roh et al, 2004 and Chang et al, 2004)*.**

The gene encoding preproadrenomedullin is termed the adrenomedullin gene and has been mapped and localized to a single locus of chromosome 11.