Functional characterization of somatostatin receptors of pancreatic insulin and glucagon-producing cells and their impact on controlling glucose homeostasis

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Vandana Singh

from Lucknow, India

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1st Reviewer: Prof. Dr. med. Bertram Wiedenmann

2nd Reviewer: Prof. Dr. Dr. h.c. Randolf Menzel

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ABBREVIATIONS

ASO	Antisense oligonucleotides
AMPK	AMP-activated protein kinase
APS	Ammonium persulphate
ATP	Adenosine triphosphate
BAT	Brown adipose tissue
BSA	Bovine serum albumin
BW	Body weight
cAMP	Cyclic adenosine mono phosphate
CBD	Common bile duct
cDNA	Complementary DNA
dsRNA	Double-stranded RNA
DEPC	Di-ethyl pyro carbonate
DIO	Diet-induced obesity
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
DMEM	Dubelcco's modified eagels medium
dNTP	Deoxynucleotide triphosphate
DPX	*p*-xylene-*bis*-pyridinium bromide
DTT	Di thio threitol
ECL	Enhanced chemiluminescence
EDTA	Ethylenediamine tetra-acetic acid
ELISA	Enzyme linked immunosorbent assay
EIA	Enzymeimmunoassay
eWAT	Epididymal white adipose tissue
FBS	Faetal bovine serum
FCS	Faetal calf serum
G6Pase	Glucose-6-phosphatase
GDP	Guanosine diphosphate
GLP-1	Glucagon like peptide-1
GPCR	G protein-coupled receptor
GcgR	Glucagon receptor
GTP	Guanosine triphosphate
iBAT	Intrascapular brown adipose tissue

IBMX	3-Isobutyl-1-methylxanthine
HBSS	Hank’s buffered saline solution
HEPES	(4-(2-hydroxy ethyl)1 piperazine ethane sulfonic acid)
dsDNA	Double stranded DNA
HEK293	Human embryonic kidney 293
HFD	High-fat-diet
HGP	Hepatic glucose production
HGP	Hepatic glucose production
KRB	Krebs-Ringer bicarbonate (KRB) buffer
LPL	Lipoprotein lipase
mAb	Monoclonal antibody
MAPK	Mitogen activated aprotein kinase
mRNA	Messenger RNA
NEFA	Nonesterified free acids
NGS	Normal goat serum
PAGE	Polyachrylamide gel electrophoresis
PAS	Periodic acid-schiff
PAb	Polyclonal antibody
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PEPCK	Phosphoenolpyruvate carboxykinase
PFA	Paraformaldehyde
PKA	Protein kinase A
PKC	Protein kinase C
PLA2	Phospho lipase A2
PLC	Phospho lipase C
PMSF	Phenyl methyl sulphonyl fluoride
RIA	Radioimmunoassay
RNA	Ribonucleic acid
RT	Room temperature
RT-PCR	Reverse transcription- polymerase chain reaction
SDS	Sodium dodecyl sulphate
SREBP	Sterol-regulated element-binding protein

SST	Somatostatin
SST2	Somatostatin receptor subtype-2
$SSTR2^{-/-}$	Homozygous deletion of sst2
ssRNA	Single-stranded RNA
ssDNA	Single stranded DNA
SSTRs	Somatostatin receptor subtypes
TAE	Tris-acetate-EDTA
TEMED	-N,N,N',N'- tetramethylethyylene-diamine
Tris	Tris (hydroxymethyl) aminomethanne
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
UV	Ultraviolet
$WT/SSTR2^{+/+}$	Wild type

Antibodies

CREB	Cyclic AMP response element binding
p-CREB	Phosphorylated cyclic AMP response element binding
FKHR	Forkhead (Drosophila) homolog 1 (rhabdomyosarcoma)
FOXO	Forkhead box, subgroup O
GAPDH	Glyceraldehyde—phosphate dehydrogenase
GCK	Glucokinase
GP	Glycogen phosphorylase
GSK-3	Glycogen synthase kinase-3
GS	Glycogen synthase
PhK	(Glycogen) phosphorylase kinase
PDK1	Phosphoinositide-dependent protein kinase 1

ABSTRACT

Somatostatin (SST) is an important regulator of insulin and glucagon secretion from the endocrine pancreas. All known SSTR subtypes (SSTR1-5) are expressed in α- and β-cells of the endocrine pancreas. In rodents, SSTR2 inhibits glucagon secretion, whereas SSTR5 inhibits insulin secretion. SSTR1, 2, 3 and 5 are expressed in human pancreas, however the role of individual SSTR subtypes in the regulation of glucagon and insulin secretion in humans is not well known. This study aims to characterize the role of SSTR subtypes in the regulation of human glucagon and insulin secretion *in vitro*. Data suggests that in humans, SST regulates both insulin and glucagon secretion mainly via SSTR2. SSTR5 is an additional receptor subtype which inhibits insulin secretion while SSTR1 inhibited glucagon secretion from human pancreas.

Postprandial (PP) impaired glucagon inhibition contributes to hyperglycemia in T2DM. In T2DM, hypersecretion of glucagon contributes to an abnormal increase in hepatic glucose production, increased rate of hepatic gluconeogenesis, thereby contributing to hyperglycemia. SST inhibits glucagon secretion in vitro mainly via receptor SSTR2 in rodents. The present study characterizes the important role of SSTR2 in the regulation of glucose homeostasis by using a mouse model of SSTR2 deficient ($SSTR2^{-/-}$) mice with high fat diet (HFD) induced obesity. Data suggests that $SSTR2^{-/-}$ mice showed increased nonfasting levels of glucose, glucagon and fasting nonesterified fatty acids levels compared to wild type (WT) mice. Islets isolated from $SSTR2^{-/-}$ mice showed an impaired inhibition of glucagon secretion by SST or glucose. Exogenous SST showed an impaired inhibition of glucagon secretion and increased levels of glucose in these animals. In addition, exogenous insulin lowered blood glucose levels less efficiently in $SSTR2^{-/-}$ mice compared to (WT) mice. Noteworthy $SSTR2^{-/-}$ mice had decreased nonfasting hepatic glycogen and lipid content. Interestingly expression and activity of enzymes regulating glycogen synthesis were decreased whereas enzymes facilitating glycogen breakdown and lipolysis were increased in $SSTR2^{-/-}$ mice. SST and SSTR2 selective agonist significantly reduced glucagon-induced glycogenolysis, without influencing *de novo* glucose production using primary hepatocytes. Taken together, these data suggest that ablation of SSTR2 in mice with HFD induced obesity leads to impaired

inhibition of glucagon secretion by glucose and SST. Increased levels of glucagon leads to impaired glucose control due to increased hepatic glycogen breakdown decreased hepatic glucose storage and less lipid accumulation.

Insulin and glucagon secretion was potently inhibited by SSTR2 selective agonist from insulin (INS-1) and glucagon secreting (InR1-G9) cells. SNX-482 (R-type Ca^{2+} channel blocker) prevented the inhibition of insulin secretion from INS-1-cells mainly via SSTR2. SSTR2 inhibited the expression of pFoxo1 and pAkt, which play the most important role in insulin secretion.

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Original articles

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