1. INTRODUCTION

1.1. Endocrine Pancreas:

The endocrine pancreas consists of four major types of small clusters of cells known as "Islets of Langerhans" **(Fig. 1)** scattered among the serous acini throughout the pancreas and are richly supplied by vessels.

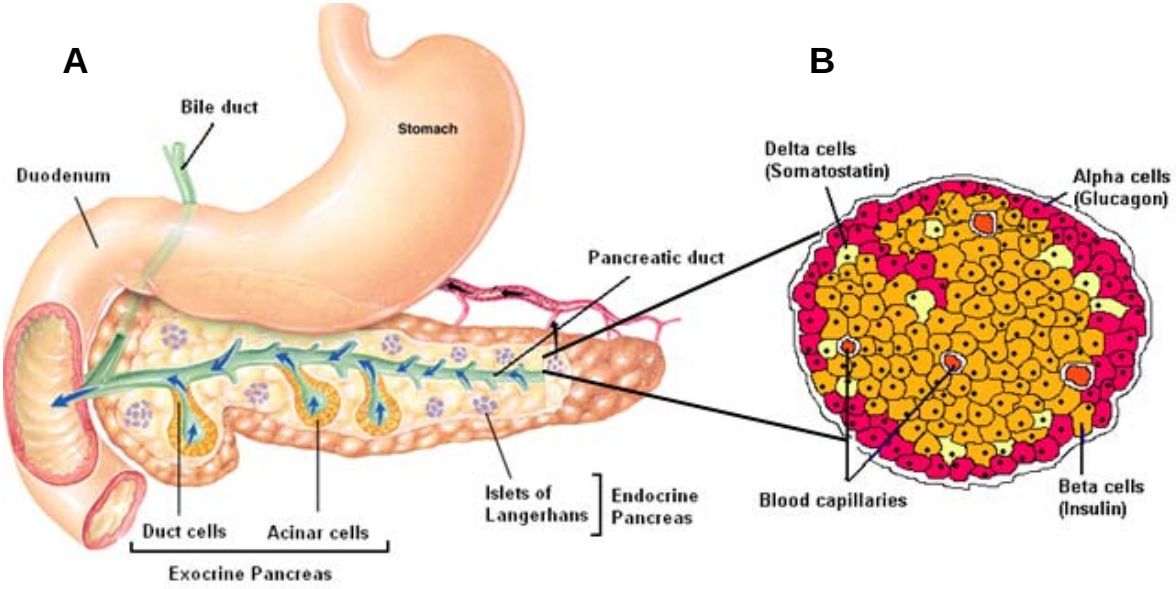

Fig. 1. Pancreas topography (A) and anatomy of the Langerhans islets (B) Adapted from: www.colorado.edu and http://cal.man.ac.uk/student_projects/2000/mnby7lc2/Islets1.gif

Paul Langerhans, a German pathologist in 1869 discovered little islands in the pancreas. In 1922 Frederick Banting and Charles Best unveiled the link between diabetes and insulin secreted by the β-cells of the pancreas (Banting & Best; 1990). The islets of Langerhans are clusters of endocrine tissue scattered throughout the exocrine pancreas. A

very small portion (approximately 2%) of total pancreatic mass makes the islets of Langerhans (endocrine part of the pancreas), which are mainly found in the tail region of the pancreas. Human endocrine pancreas consists of approximately one million islets. Each islet is consists of approximately 3000 cells.

The endocrine pancreas comprises four principal cell types, which are also derived from the endoderm, α-cells, β-cells, delta δ-cells and polypeptide cells (PP) (Patel; 1999)**.** Endocrine cells of the rodent islets show a specific distribution pattern. **A or α-cells** which make 10% of the total cells in islets are found in the periphery of the islet and are responsible for the secretion of glucagon. **B or β-cells** makes approximately 70% of endocrine cells and are located centrally. β-cells are responsible for insulin secretion. The importance of the anatomic distribution has been demonstrated before. If islets are dispersed into single cells, they reassemble again to form a primitive islet-like structures with beta-cells in the centre and other islet cells in the periphery (Halban et al., 1987). **D or δ -cells** which are responsible for the secretion of somatostatin (SST) are bigger in size and commonly found in the periphery of the islets along with α-cells. SST-producing cells make up 5% of all hormonally active cells found in the islets. Approximately 15% of endocrine active cells of the pancreatic islets are the PP cells, (Ballian; 2006)

1.1.1. Development and differentiation of endocrine cells of the pancreas

Beside overeating and life style diabetes is also a multifactorial disease which is caused by the alterations in several gene products. The development of pancreas and as well as endocrine cells is orchestrated by number of genes.

At the molecular level, the differentiation of the islet cells and pancreatic development depends on the interaction of multiple transcription factors, signalling molecules and growth factors. During organogenesis, transcription factors regulate the differentiation of endocrine and exocrine cells, production of hormone and gene expression of the pancreas. (Sander & German; 1997) (German et al., 1995) (Ballian; 2006) **(Fig. 2a)**

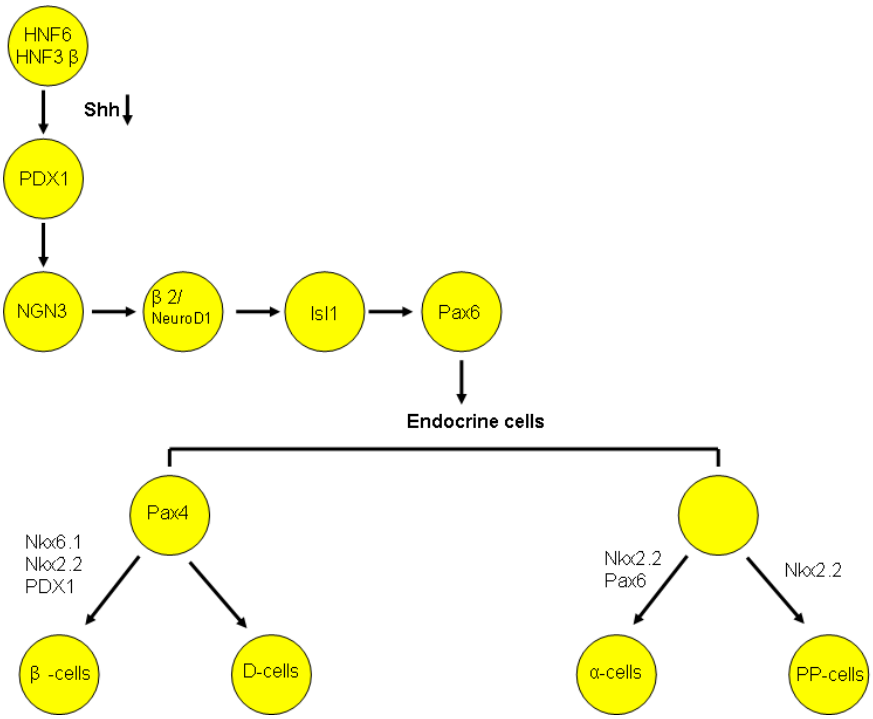

Fig. 2a. Representation of signalling and transcription factors involved in endocrine pancreatic development.

Adapted and modified from Habener et al., 2005; *Endocrinology*

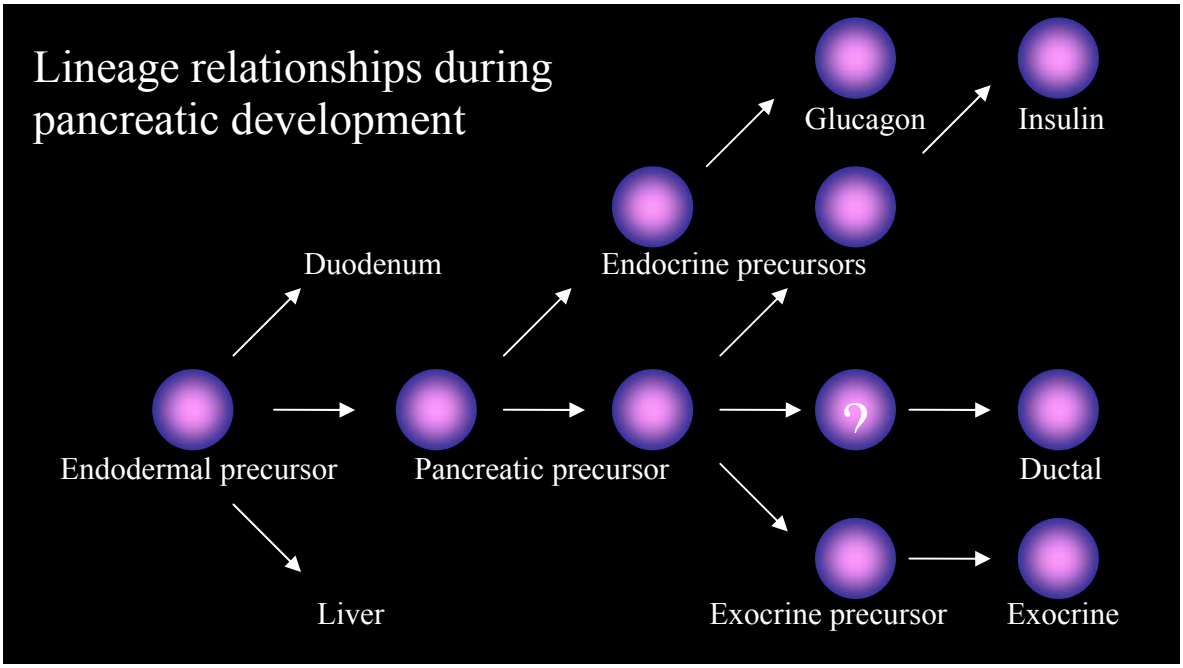

Fig. 2b. Representation of endocrine and exocrine pancreatic development
Adapted from www.uchsc.edu/misc/diabetes/xch2af6.gif

The pancreas is derived from the outpoketings of endoderm from the primitive gut. It develops from the dorsal and ventral outgrowth of the primitive duodenum. Recent studies have investigated that the endocrine and exocrine pancreas originated from a common cell (Fishman & Melton; 2002) **(Fig. 2b)**.

Several transcription factors are involved in endocrine and exocrine development of the pancreas, which are expressed in a definite patterns.

The family of (HNFs) (Hepatocyte nuclear factors) plays a key role in endocrine cell development of the pancreas. Expression of Hnf3β (also known as Foxa2) is induced by Hnf6, which regulates the expression of Pdx1. For the development of β-cells, differentiation occurs in the adult pancreas. Pdx1 expression is found during early pancreatic development and also in the mature β-cells. Previous studies have revealed that Pdx1 regulates the expression of insulin, (Ohlsson et al., 1993) glucokinase (GK)

enzyme (Watada et al., 1996) and glucose transporter type 2 (GLUT2) (Waeber et al., 1996) in adult β-cells.

The Pax (paired-homeodomain transcription factor) family also plays a key role in pancreatic development and islet cell differentiation of which Pax6 is required for the development of all endocrine cells of the islet. Recent studies shows that the animals in which Pax6 gene was knocked out lacked proper islet morphology and showed reduction in α and β cell secreted hormones (Sander & German; 1997) (St-Onge et al., 1997). Pax4 transcription factor regulates the formation of β- and δ -cells in endocrine pancreatic development. Pax4 deficient mice lacks β- and δ -cells in islets but the number of α-cells are increased (Sosa-Pineda; 1997). Of note, Pax4 inhibits glucagon gene expression via Pax6 by DNA binding competition (Ritz-Laser et al., 2002).

Development of any organism or the secretion of any hormone is basically regulated by the genes and by understanding the cascade of gene regulation events. This could be possible to provide new insights into the inherited defects of diabetes and insulin deficiency.

1.2. β -cell function

The main function of the islet β-cell is the controlled synthesis and release of insulin in response to various nutrients, gastrointestinal and pancreatic hormones and autonomic nervous stimulus to maintain the normal concentration of blood glucose levels for the proper functioning of the tissues. In healthy individuals at hyperglycemia (e. g. after food intake) insulin secretion from the β-cells rises, whereas glucagon secretion decreases. Insulin stimulates glucose uptake in liver, muscle and fat (anabolic process). During hypoglycemia or in fasting state the secretion of insulin declines whereas glucagon secretion increases. The consequence is a stimulation of hepatic glucose production and inhibition of glucose uptake by tissues from the circulation.

Insulin (a 51 amino acid peptide) is synthesized as a 110 amino acids single-chain precursor molecule preproinsulin. In the rough endoplasmic reticulum, it is further processed to proinsulin which is transferred to golgi apparatus and finally to secretary granules where it is processed to mature insulin by the action of two prohormone convertase enzymes PC1/3 and PC2. Synthesized insulin is stored in dense-core granules

It has been identified that the blood flows from β-cells towards the α and δ -cells (Samols et al., 1986). Insulin regulates the secretion of neighboring cells by auto/paracrine as well as endocrine interactions (Ishihara et al., 2003).

Insulin secretion is a dynamic process; it begins when glucose enters in the cell through glucose transporting proteins e.g. GLUT2 in pancreatic β-cells and liver cells or GLUT4 in adipose tissue and skeletal muscle. Then glucose is phosphorylated by an enzyme glucokinase. This leads to a change in ATP/ADP ratio, closes the ATP sensitive potassium channels and causes depolarization in the cell membrane.

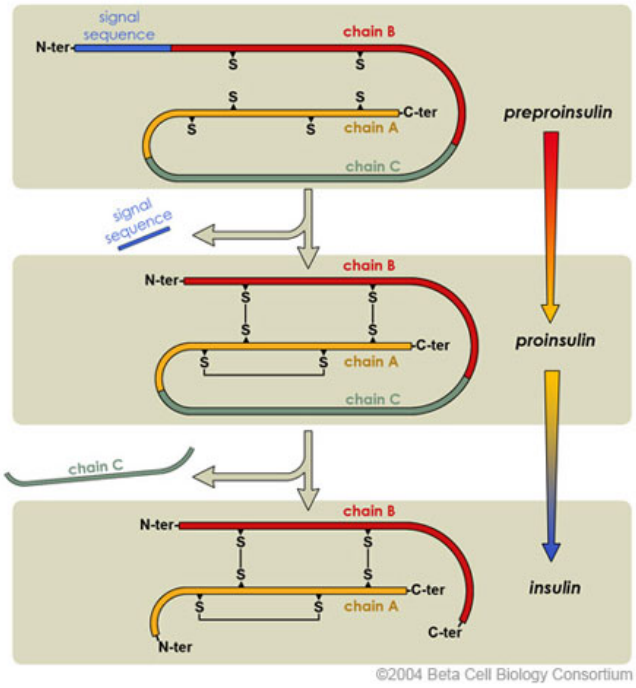

Fig. 3. Schematic representation of insulin synthesis
Adapted from www.betacell.org

The result is a calcium influx into the cell, which finally activates the insulin release (Henquin J-C; 2000) **(Fig. 4)**. GLUT4 and glucokinase act as glucose sensors and the transport of glucose into the β-cells are quite active at higher glucose levels i.e. after food intake.

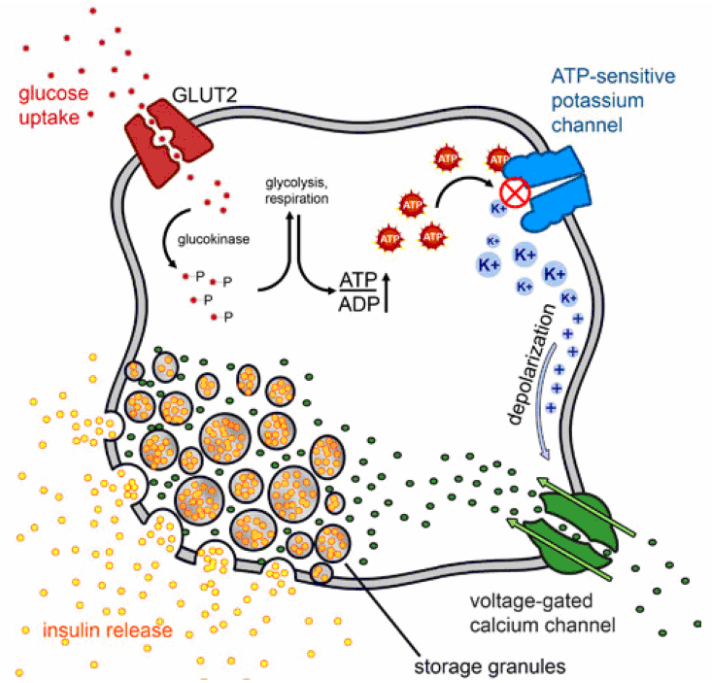

Fig. 4. Mechanisms in insulin release from β-cells.
Adapted from www.betacell.org

1.2.1. β-cell signaling

β-cell expresses receptors for insulin and growth factors (for e.g. IGF-1, EGF etc.). The ligand-activated receptor initiates the cascade by phosphorylating the family of IRS (insulin receptor substrates 1-4), which serves as the key protein for the further events of the signaling cascade (e.g. PI3kinase). The heterodimer protein further stimulates other kinases, like protein kinase B (Akt/PKB). Mitogen-activated protein (MAP) kinase cascade is activated by Ras oncoprotein. Activated Ras binds to Raf-1, which is a serine/threonine protein kinase activates MAP kinase cascade. PI3 kinase plays a central role in metabolic actions of insulin because inhibition of PI3 kinase pathway blocks the metabolic activity of insulin e.g. glucose transport, glycogen synthesis and lipid synthesis (Saltiel et al., 2001) **(Fig. 5)**.

Recent studies have demonstrated the presence of AMP-activated protein kinase in the β-cells of the pancreatic islets (Da Silva Xavier et al., 2000) (Da Silva Xavier et al., 2003).

Its central role in the metabolism of carbohydrate and fat has been demonstrated by Winder at al (Winder et al., 1999) in response to change in ATP/ADP ratio in the β-cell. AMPK is a heterotrimeric serine/threonine protein kinase, which inactivates/phosphorylates several metabolic enzymes involved in fatty acid synthesis when activated, suggesting a very important role in the regulation of insulin secretion. These studies also demonstrated that AMPK might have a role in the regulation of pre-pro-insulin promoter.

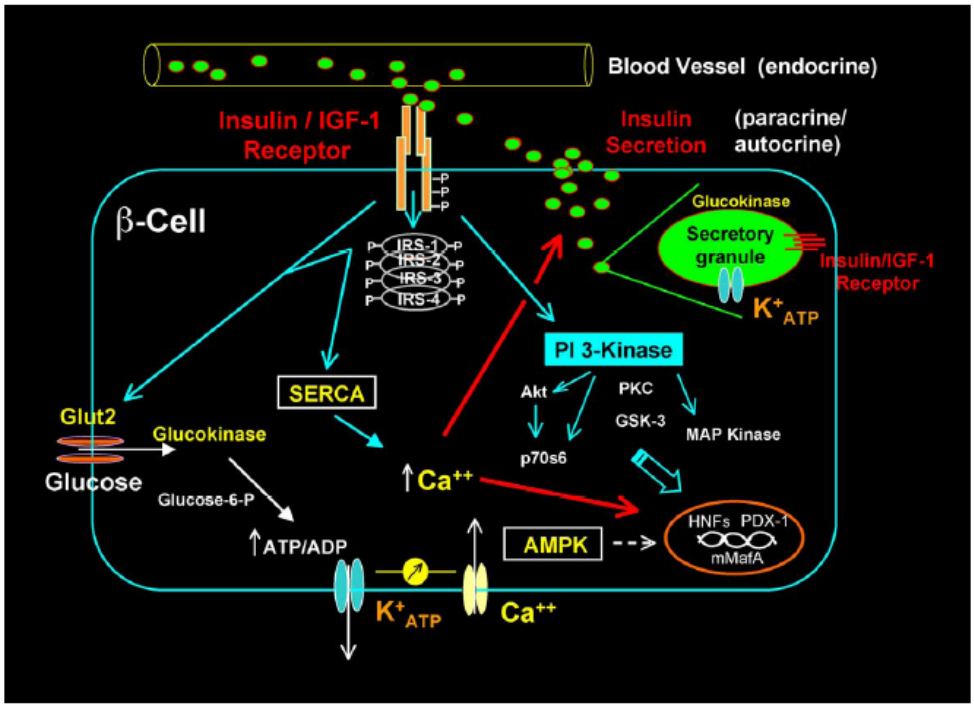

Fig. 5. Insulin signaling in β-cells of the endocrine pancreas.
Adapted from Kulkarni; 2004. *Int J Biochem Cell Biol*

The important target tissues for insulin are liver, muscle adipose tissue and brain. Insulin is the primary hormone responsible for the control of glucose utilization in the body and

also the storage for further utilization. Insulin promotes the anabolic processes and inhibits catabolic processes for e.g. breakdown of glycogen, fat and protein.

1.2.2. β-cell dysfunction and impaired insulin action in T2DM

Diabetes mellitus is a multifactorial disease, which results with the combination of genetic factors and the factors, which impairs insulin secretion or β-cell function, and/or insulin resistance (Gerich; 1998). Insulin controls the blood glucose levels in the body and maintains the blood glucose level to normal which is achieved by the balanced insulin secretion and action. T2DM occurs when there is β-cell dysfunction due to abnormalities in insulin secretion and action and various degrees of insulin resistance as detected by impairment of glucose transport in skeletal muscle and adipose tissue in response to treatment with insulin.

Obesity occurs due to insulin resistance. Factors like high fat diet, weight gain and/or lack of exercise can lead to obesity in particularly in those individuals who have genetic predisposition. Elevated levels in free fatty acids (FFA) and chronic hyperglycemia can lead to β-cell dysfunction. Rise in FFA results from increased lipolysis in adipocytes. Increased FFA levels in the circulation further contribute to insulin resistance. (Boden G; 1996).

Delay in acute insulin response to glucose is an early indication of β-cell deterioration (impairment of the first-phase of insulin secretion). In normal individuals the first phase of insulin response to glucose begins immediately and reaches to its peak within 10 min and disappears within 20 min. The second phase of insulin response begins in 15-20 min and reaches to its peak in 20 to 40 min. In type 2 diabetic patients, there is lack of first phase of insulin response to glucose.

Loss in β-cell function is genetically predetermined. It has been demonstrated that β-cell loss may result from insulin resistance, glucotoxicity (Kaiser et al., 2003) and lipotoxicity (Unger; 1995) (Robertson et al., 2004). Although β-cell deterioration due to glucotoxicity and lipotoxicity can be partially reversed by restoration of metabolic control the progressive β-cell loss can not be stopped despite constantly increasing spectrum of antidiabetic agents.

1.3. α-cell function

α-cells of the endocrine pancreas secrete glucagon, which plays a key role in maintaining glucose homeostasis. It is the major counterpart of insulin and is released into the blood circulation during hypoglycemia. Glucagon can stimulate hepatic glucose production by facilitating glycogenolysis and gluconeogenesis to encompass hypoglycemia.

The secretion of glucagon within the islets is a tightly regulated process. Within the enodcrine pancreas, the secretion of glucagon is controlled by insulin and somatostatin secreting cells. Similar to insulin, secretion of glucagon is also regulated by the nutrients, hormones and autonomic nervous system. (Pipeleers et al., 1985).

Glucose and fatty acids inhibit glucagon secretion, whereas cholecystokinin and activation of both sympathetic and parasympathetic nervous system stimulate glucagon secretion. At low plasma glucose concentration or in the presence of amino-acids (arginine) glucagon secretion increases.

Glucagon is a single-chain polypeptide consisting of 29 amino acids which is synthesized from a large precursor molecule proglucagon. Proglucagon is processed to mature glucagon by prohormone convertase 2 (PC2).

1.3.1. α-cell dysfunction in T2DM

In diabetic patients, there is presence of abnormal function of not only beta-cells but also uniform abnormality is found in α-cells. In normal individuals, α-cells secrete glucagon during fasting state. Glucagon restores normoglycemia by the breakdown of stored macromolecule glucose such as hepatic glucose. Noteworthy, in diabetic patients α-cells often fail to respond hypoglycemia.

Physiologically, a rise in glucose levels leads to a suppression of glucagon secretion and stimulates insulin secretion. A completely different situation is found in type 2 diabetes. Up to 70% of patients with T2DM have impaired inhibition of glucagon secretion despite high blood glucose levels (Shah et al., 1999). Glucose not only fails to inhibit glucagon secretion, even a paradoxical increase in plasma glucagon level is observed in patients with T2DM. The result is deterioration of hyperglycemia. (Unger et al., 1970) (Ohneda et al., 1978) (Dinneen et al.1995). Patients with T2DM have impaired suppression of hepatic glucose production in response to increase in glucose levels at postprandial state.

Furthermore, an excessive stimulation of alpha-cells after the infusion of amino-acid (arginine) (Unger et al., 1970) (Raskin et al., 1976) or protein meal compared to non-diabetic individuals has been reported (Gerich et al., 1975).

1.4. Somatostatin (SST)

Somatostatin (SST) is a neuropeptide originally isolated from the hypothalamus as a inhibitor of growth hormone secretion. (Krulich et al., 1968). SST is a multifunctional peptide secreted by a number of tissues, including pancreas, intestinal tract, thyroid, adrenals and kidneys and regions of the central and peripheral nervous system (Lars et al., 2003) (Patel et al., 1995) (Reichlin S; 1983). SST is also produced by inflammatory and immune cells where it is responsible for T-cell proliferation, lymphocyte adhesion and cytokine production. SST is also secreted by many cancer cells.

The secretion of SST is influenced by nutrients, neurotransmitters, hormones, ions, growth factors and cytokines. Glucagon stimulates both insulin (Samols et al., 1965) and SST secretion by acting on glucagon receptors present on beta- and delta-cells, whereas insulin inhibits both glucagon (Samols et al., 1976) and SST secretion. (Patel et al., 1982) (Samols et al.,1990). Within the pancreatic islet, SST inhibits both insulin and glucagon secretion. (Brunicardi et al., 2001) (Brunicardi et al., 2000). Thus, the secretion of the cells present in the islet can influence the secretion of each other by auto/paracrine action **(Fig. 6)**.

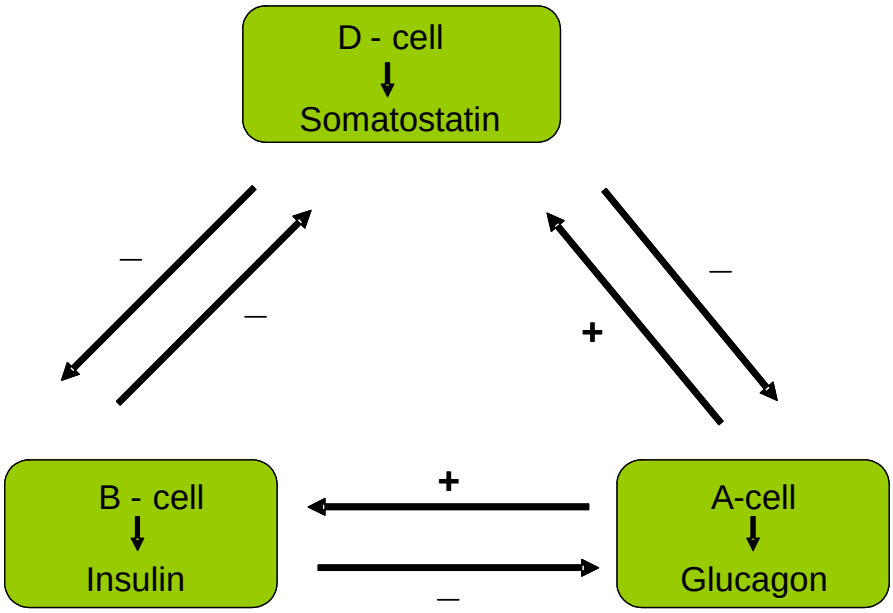

Fig. 6. Intra-islet interactions between A (α-), B (β-) and D (δ-) cells of the endocrine pancreas

1.4.1. Somatostatin synthesis

There are two most important biologically active isoforms of SST, SST-14 and SST-28, reflecting their amino acid chain length. In humans, SST-14 and SST-28 are encoded by only one gene. SST is synthesized from a 116-amino-acid peptide, preprosomatostatin by proteolytic cleavages (Funckes et al., 1983). In mammals, preprosomatostatin is encoded by a single mRNA sequence (Montminy et al., 1984). By proteolytic cleavage of 24 amino acids at the N-terminus of preprosomatostatin leads to formation of prosomatostatin (Noe et al., 1989) Prosomatostatin is processed in two different ways to generate SST-14 and SST-28 (Montminy et al., 1984) in a tissue specific manner. SST-14 is synthesized in brain, pancreas and gastric tissue while SST-28 is mainly synthesized in the intestinal epithelium. The half life of SST-14 is 2 to 3 min while that of SST-28 is slightly longer due to it greater metabolic stability. SST is cleared from the body mainly by liver and kidneys. (Polonsky et al. 1981)

In addition to tissue-specific differences in secretion of SST-14 and SST-28, the two forms of this hormone can have different biological potencies. SST-28 is roughly 10-fold more potent than SST-14 in inhibiting growth hormone secretion, but less potent in inhibiting glucagon release.

1.5. Somatostatin receptor subtypes (SSTRs)

1.5.1. Morphology of SSTRs

The biological effects of SST are mediated through a family of G protein coupled receptors. They comprise seven transmembrane domains (TMD) **(Fig. 7)**. Five SSTRs (SSTR1- SSTR5) have been cloned from various species including human, rat and mouse (Yamada et al. 1992) (Yamada et al., 1993).

The length of SSTRs sequence in humans is between 364 - 418 amino acid residues. The highest degree of sequence identity is seen in TMDs (Bell et al., 1995). SSTRs can be classified into two groups according to the structural information (sequence homology), SSTR1/ SSTR4 group and SSTR2/ SSTR5/ SSTR3 group and the sequence similarity exists in TMDs. Human SSTRs are encoded by five different genes located on different chromosomes. Except SSTR2 gene SSTRs are intronless. SSTR2 is spliced to form two isoforms called SSTR2A and SSTR2B, which differ in their C-terminal. (Lahlou et al., 2004).

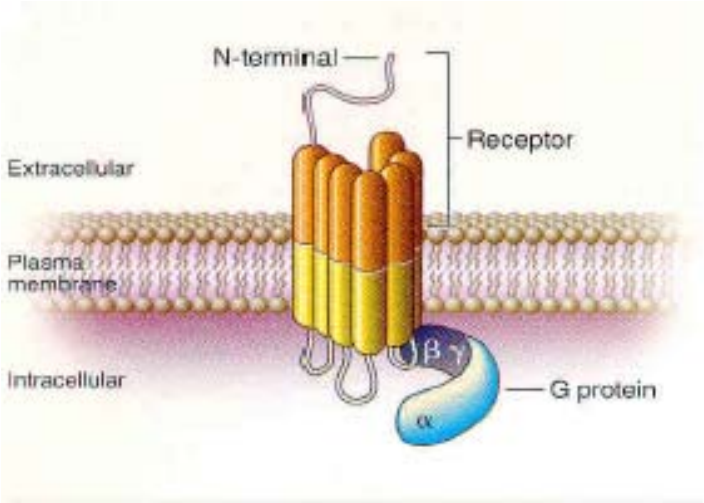

Fig. 7. Structure of SST receptor showing intracellular and extracellular domains. Adapted from Lamberts et al., 1996; *N Engl J Med.*

All the SSTRs show high affinity to both SST-14 and SST-28. However, SSTR5 shows approximately 6-fold higher affinity to SST-28 compared to SST-14 (Patel, Srikant; 1994) (Patel et al., 1995) (Patel et al., 1996).

1.5.2. Intracellular signalling

Intracellular signalling is complex process, which starts by the binding of SST or SST analogues to different SSTRs. This activates G-protein and induces signalling via several transduction pathways. Each SSTR is coupled to multiple intracellular transduction pathways **(Table 1)** via pertusis toxin-sensitive and insensitive heterotrimeric GTP-binding G proteins. All five SSTRs are negatively coupled to adenylate cyclase i.e activation of the receptor results in inhibition of adenylate cyclase. Upon binding of SST a fall of intracellular cAMP levels occurs. All five SSTRs stimulate phopholipase C and Ca^{2+} mobilizations via both pertusis toxin-sensitive and insensitive G proteins (Benali et al., 2000). Activation of SSTRs results in membrane hyperpolarization by regulating activation of K^+ channels, which lead to decreased Ca^{2+} influx through voltage-gated Ca^{2+} channels and reduction in intracellular Ca^{2+}. SSTRs are also involved in the signalling of MAP (mitogen activated protein) kinase pathway but varies with the receptor subtypes.

SSTR1 stimulates Na^+/H^+ exchanger via pertussis toxin sensitive mechanism, while SSTR1 and SSTR4 activates MAP kinases Erk1/2 cascade via pertussis toxin sensitive G-proteins in CHO-K1 cells (Bito et al., 1994) (Florio et al., 1999). Although SSTR5 inhibits MAP kinase cascade by inhibiting guanylate cyclase and cGMP dependent protein kinase G (Cordelier et al., 1997). SSTR1, 2, 3 and 4 stimulated the activity of tyrosine phosphatase in NIH 2T3 and CHO cells (Buscail et al., 1994) (Reardon et al., 1997). In addition, SSTR2 inhibits Ca^{2+} entry in RIN 5F cells by inhibiting voltage-dependent Ca^{2+} channels (Fujii et al., 1994). **Table 1** summarizes some of the properties of individual SSTRs.

Table 1. Intracellular signalling coupled to each SSTRs

	sst1	sst2	sst3	sst4	sst5
Transduction pathway					
Adenylate cyclase	↓	↓	↓	↓	↓
Phospholipase C	↑	↑	↑	↑	↓/↑
Tyrosine phosphatase	↑	↑	↑	↑	↔
Ca^{2+} channels	↓	↓			
K^+ channels	↔	↑	↑	↑	↑
Na^+/H^+ exchange	↓	↔			
MAP kinase (ERK)	↑			↑	↓
Phospholipase A2				↑	

Adapted from Benali et al., 2000; *Digestion*

Original data from (Bruns et al., 1995)

1.5.3. Expression and functions of SSTRs

SSTRs are widely distributed throughout many tissues including brain, gut, pituitary, endocrine and exocrine pancreas, adrenals, thyroid, lungs, kidneys, inflammatory, immune and tumor cells. SSTRs show a tissue and species specific expression pattern (Yamada et al., 1992).

SSTRs are involved in different physiological processes, e.g. neurotransmission, inhibition of gastrointestinal motility, gastric acid flow, intestinal absorption, pancreatic enzyme secretion, GH release and cell proliferation.

In the endocrine pancreas, immunohistochemical studies demonstrated SSTR2 expression predominantly in α-cells and SSTR5 in β-cells. It has been suggested that in rodents SSTR2 is responsible for glucagon inhibition while SSTR5 for insulin secretion.(Mitra et al., 1999). This has also been identified by *in vitro* studies on isolated pancreatic islets in rodents (Fagan et al., 1998) (Cejvan et al., 2003) (Strowski et al., 2003) (Strowski et al., 2000).

1.6. Somatostatin ligands

SST has a wide range of physiological functions. Therefore, it plays a pivotal role in many human diseases. However, the clinical utilization of SST has been restricted because of very short half life in the circulation and lack of selectivity to the specific SST receptors. To overcome these problems, SST analogues (SST peptide agonist and antagonists, non-peptide agonists and antagonists and radioligands or SST conjugates) (Weckbecker G. et al., 2003) with much more stability and selectivity have been developed.

1.6.1. Somatostatin agonists

Octreotide/sandostatin (SMS 201-995) is a cyclic octapeptide, used clinically to treat patients suffering from acromegaly and carcinoid tumors. Octreotide shows high affinity binding with SSTR2, along with intermediate affinity binding to SSTR3 and SSTR5 (Weckbecker G. et al., 2003). Nowadays, lanreotide (BIM-23014) (Murphy et al., 1987) and vapreotide (RC-160) which exhibits the greater metabolic stability and selectivity to the SST receptors are used clinically (Lamberts et al., 2002). By further modification in the structure, other SST analogue, MK-678 and L-363,301 were synthesized. They showed greater affinity binding to SSTR2 and approximately 10-fold higher potency than the native SST (Janecka et al., 2001).

SOM-230, a cyclohexapeptide also known as the universal ligand binds with high affinity to SSTR1, SSTR2, SSTR3 and SSTR5 and with a lower affinity to SSTR4. It is a potent inhibitor of growth hormone/IGF-1 (insulin growth factor-1) release and has the half life of 24 hours (Bruns et al., 2002).

KE108 is another analogue, which binds with nanomolar affinity to all SST receptors and shows longer stability in circulation. It inhibits cAMP production (Reubi et al., 2002). L-362,855 is a larger metabolically stable analogue like KE108 and shows high binding affinity to SSTR2, SSTR3 and SSTR5 due to it modified structure. BIM23268 and BIM23052 are the peptide analogues of SST, which exhibits selectivity towards SSTR5 receptor subtype (Shimon et al., 1997).

Discovery of the non-peptide agonists resulted to a promising and novel alternative for peptide analogues. Recently by the use of combinatorial chemistry, discovery and

synthesis of selective non-peptidal agonists is in progress. L-054,264 and L-054,522 are the two non-peptide agonists, which have been synthesized by the combinatorial chemistry methods. Both of these agonists show high selectivity to SSTR2 subtype. Other selective non-peptide agonists had also been reported e.g. SSTR2 selective L-779,976, which inhibits growth hormone and glucagon secretion in rodents. SSTR4 selective L-803,087 and SSTR5 selective L-817,818 also inhibits insulin secretion from the mouse islets (Rohrer et al., 1998) (Rohrer et al., 2000). The structure of relevant nonpeptidal SSTRs selective agonists used in our study are mentioned in **(Fig. 8)**.

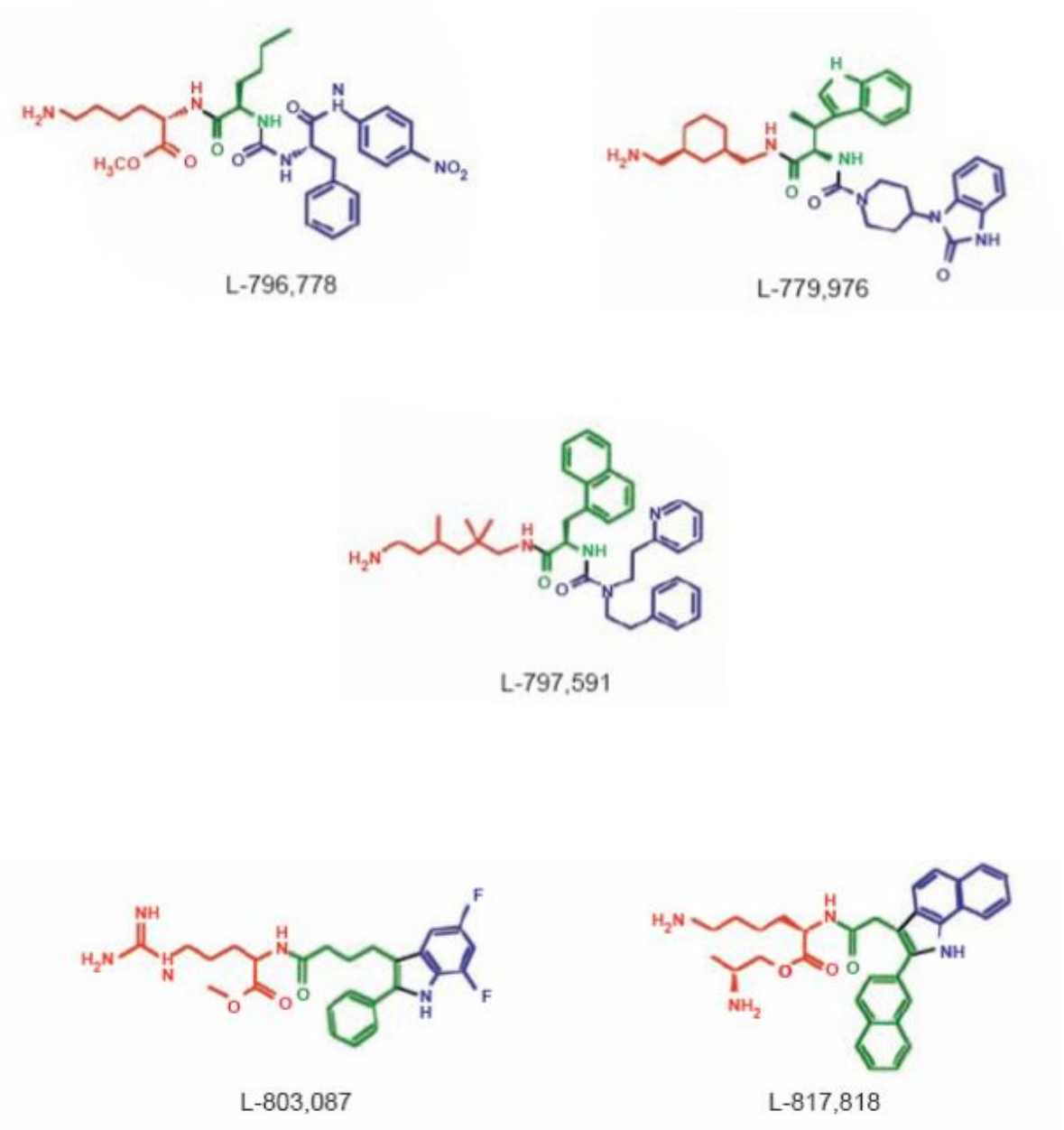

Fig. 8. Structure of SSTRs nonpeptide agonists where L-796,778, L-779,976, L-797,591, L-803,087, and L-817,818 are selective agonists for SSTR1, SSTR2, SSTR3, SSTR4 and SSTR5 respectively.

Adapted from Rohrer et al., 1998; *Science*

1.6.2. Peptidal somatostatin antagonists

CYN-154806 was the first peptide antagonist specific for SSTR2 (Bass et al., 1996). PRL-2970 is another peptide antagonist, which binds to SSTR2 receptor and is the most potent antagonist known till date. Bass et al., found that modification of a weak agonist by the inversion of chirality at positions 5 and 6 leads to comparative antagonist. This was later explored by the other group (Hocart et al. 1998).

Other selective peptide antagonists known are BIM-23627 (Tulipano et al., 2002), BIM-23056 (Wilkinson et al., 1996) and SSTR3-ODN-8 (Reubi et al., 2000). This showed selectivity towards SSTR2, SSTR5 and SSTR3 respectively.

SRA-880 (Hoyer et al., 2004) and BN-81674 (Poitout et al., 2001) are SSTR selective non-peptide antagonist. SRA-880 shows selectivity to SSTR1 receptor while BN-81674 shows highest affinity for SSTR3 receptor. SRA-880 reduces aggressive behaviour in mice without any side effects (impairment of locomotion). However, these new synthesized peptide as well as non-peptide antagonists needs to be further analyzed because some of them might work as agonists depending on type of tissue.

1.7. SSTRs of the endocrine pancreas: expression and function

1.7.1. Expression of SSTRs in rodents

The expression of SSTRs varies with sex, age, organ or tissue and species (Ballian et al., 2006). Bruno et al., have shown that all five SSTRs are expressed in CNS, pituitary and spleen (Bruno et al., 1993). Tissue specific distribution of SSTRs was identified by RT-PCR in peripheral tissues and CNS of rat (Raulf et al., 1994). This study showed the expression of all SSTRs in brain and pituitary, although lungs, liver and pancreas showed the variation in expression of SSTRs. Later on, other groups detected the mRNAs of all five SSTRs in the brain (Fehlmann et al., 2000) and retina (Cristiani et al., 2002) of mouse. Although kidney express only SSTR3, SSTR4 and SSTR5 (Bates et al., 2003).

Table 2. Tissue specific expression of SSTRs at mRNA levels in rodents

Expression of SSTRs	Tissue specific mRNA expression in rodents
SSTR1 - SSTR5	CNS, pituitary and spleen of rats
SSTR1 - SSTR5	Brain and retina of mouse
SSTR1 - SSTR5	Pancreatic islets of rats and mice
SSTR3, SSTR4 and SSTR5	Kidney of mouse

Ludvigsen et al., have demonstrated the expression of all SSTRs in pancreatic α- and β-cells of rodents (Ludvigsen et al., 2004). This study was done on the pancreatic islets of rats and mice using antibodies against SSTR1- SSTR5 receptors. The hormones secreted by the cells of the endocrine pancreas (insulin, glucagon, somatostatin and polypeptide) to investigate the co-localization of SSTR1-5 in the pancreatic islet cell in rodents.

1.7.2. Functions of SSTRs in rodents

It has been demonstrated that the expression pattern follows the functional pattern of SSTRs in rodents. Studies have shown that in rodents SSTR2 is more potent inhibitor of glucagon secretion and SSTR5 of insulin secretion both *in vivo* and *in vitro* (Rossowski and Coy; 1994) (Fagan et al., 1998) (Strowski et al., 2000) (Cejvan et al., 2003).

Recently, (Strowski et al., 2006) have demonstrated in animal model of type 2 diabetes, that even in postprandial state both circulating glucagon and glucose levels were decreased with highly selective non-peptidal SSTR2 selective agonist (compound 1). This study also showed the inhibition of glucagon secretion from isolated pancreatic islets from wild type animals treated with compound 1. This further confirms the important role of SSTR2 in regulation of glucagon secretion.

1.7.3. Expression of SSTRs in humans

In humans, immunohistochemical studies have identified that all SSTRs are expressed in α- and β-cells in human pancreatic islets (Portela et al., 2000), with high expression of SSTR1, SSTR2 and SSTR5 whereas low expression of SSTR3 and SSTR4 (Kumar et al.,

1999) **(Fig. 9)**. The expression pattern varies with endocrine cell types. For example β-cells express SSTR1 and SSTR5, α-cells express SSTR2 and δ-cells express SSTR5 (Kumar et al., 1999). Reubi at al., have found the expression of SSTR2 on both α- and β-cells in human pancreatic islets by using different antibodies (Reubi et al., 1998).

Table 3. Tissue specific expression of SSTRs at protein levels in humans

Expression of SSTRs	**Tissue specific expression of SSTRs at protein levels in humans**
SSTR1 -SSTR5	Pancreatic A-and B-cells

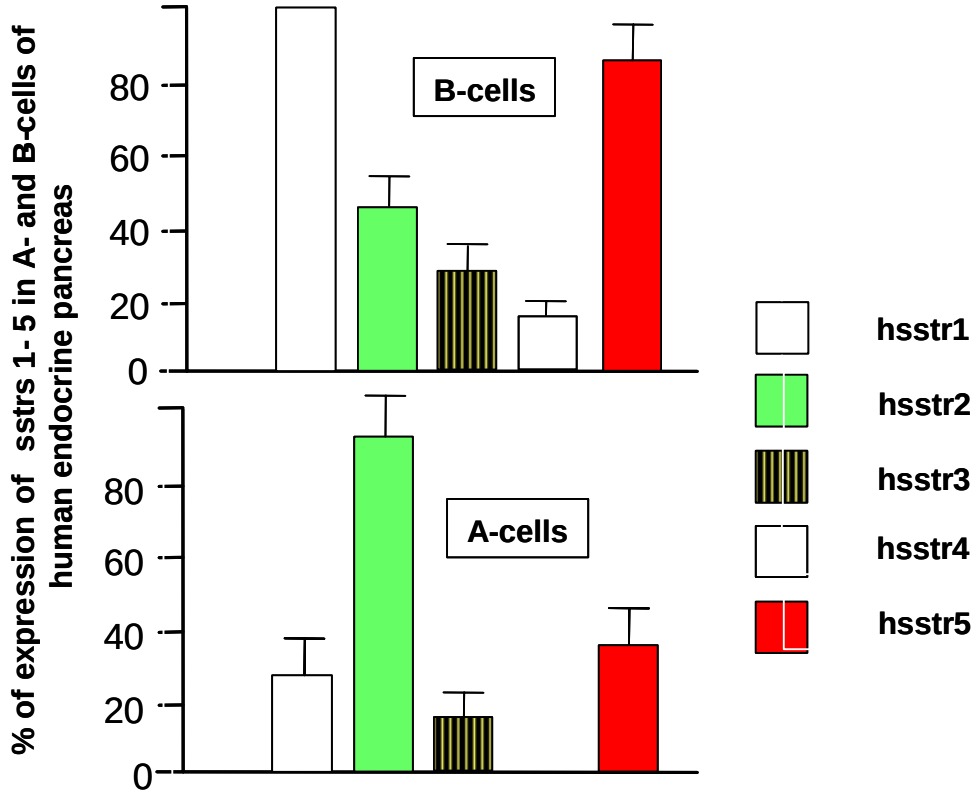

Fig. 9. Expression of SSTRs 1-5 in A and B- cells of the human endocrine pancreas (hsstr = Human SSTR).

Adapted from Kumar et al., 1999; *Diabetes*

1.7.4. Functions of SSTRs in humans

In humans there are not only the discrepancies in expression of SSTRs but also in functions. Atiya et al., reported that SSTR2-selective agonist inhibits insulin secretion from perfused human pancreas, whereas SSTR5- and SSTR3-selective agonists had no effects (Atiya et al., 1997). Moldovan et al., reported a decrease in insulin secretion from perfused human pancreas using a single dose of an SSTR2- selective agonist DC32–87 (Moldovan et al., 1995). Zambre et al., identified a dose dependent inhibition of insulin secretion from isolated human pancreatic islets by using moderate SSTR5 selective

agonist, while no effect with SSTR2 selective agonist on insulin secretion was observed (Zambre et al., 1999). Brunicardi et al., demonstrated that SSTR2 selective agonist inhibited insulin secretion in isolated perfused human pancreas, although no effect with SSTR3 and SSTR5 was registered (Brunicardi et al., 2003).

Further studies have shown that in humans, α- and –β cells of the endocrine pancreas express SST receptors. SSTR1, SSTR2, SSTR3 and SSTR5 are expressed in human pancreas. However, contribution of individual receptor subtypes in the regulation of glucagon and insulin secretion is not well known.

1.8. Role of SSTRs analogues in pathophysiology of T1&T2DM

Within the islets, SST secreting cells lie at the periphery with close proximity with α-cells and constitute approximately 10% of the total islet cell mass. According to the cell type location within the islets, it suggests that endogenous SST has primary affect on glucagon secretion than on insulin secretion (Orci & Unger; 1975). δ -cells mass and SST secretion from the endocrine pancreas is altered in T1DM (Type 1 diabetes). Similar effects were found in streptozotocin induced diabetic rats (Patel & Weir; 1976**).** These studies suggest a possible role SST in pathogenesis of diabetes (Unger et al., 1977).

SST has a very short half life period and binds to all five SSTRs with similar affinity. Therefore, synthetic SSTRs analogues were developed for the clinical use with longer stability. However, the SSTRs have insufficient selectivity and different binding affinities with each SSTR subtypes. SOM230 is the only analogue, which binds to all SSTRs except SSTR4 with equal affinity (Bruns et al., 2002).

In theory, SST and its analogues can be used clinically for the treatment of diabetes because SST inhibits the secretion of hormones (glucagon and growth hormone), which can lead to the improvement of the metabolic disturbances. It was speculated that abnormality in the secretion of SST can lead to the complications of diabetes (Gerich et al., 1990).

Growth hormone plays an important role in glucose metabolism (Luft R & Cerasi E; 1964). Abnormal increased levels of GH is found in T1D individuals (Hansen AP & Johansen K; 1970). Hypoglycaemia is observed in such patients and GH replacement brings the plasma glucose levels to normal by increasing hepatic glucose production

(Bougneres et al., 1985). GH stimulates lipolysis, which results in increase in FFAs and glycerol as substrates for energy metabolism. GH stuimulates liver gluconeogenesis and inhibits insulin induced inhibition of hepatic gluconeogenesis (Rizza et al., 1982).

In T1DM patients there is an abnormal excess secretion of GH (Hansen & Johansen; 1970). This leads to abnormal glucose metabolism. The change in glucose metabolic control occurs due to reduction in insulin sensitivity (Bratusch-Marrain et al., 1982). Diabetic retinopathy is the most common micro-vascular complication of diabetes that effects the blood vessels of retina which leads to blindness among working age individuals **(Fig. 10)** (Kohner; 1993). SSTRs are also expressed in the retina and clinically octreotide is used for the treatment of diabetic retinopathy. This may reduce the need for surgery (Grant et al., 2000) (Boehm et al., 2001).

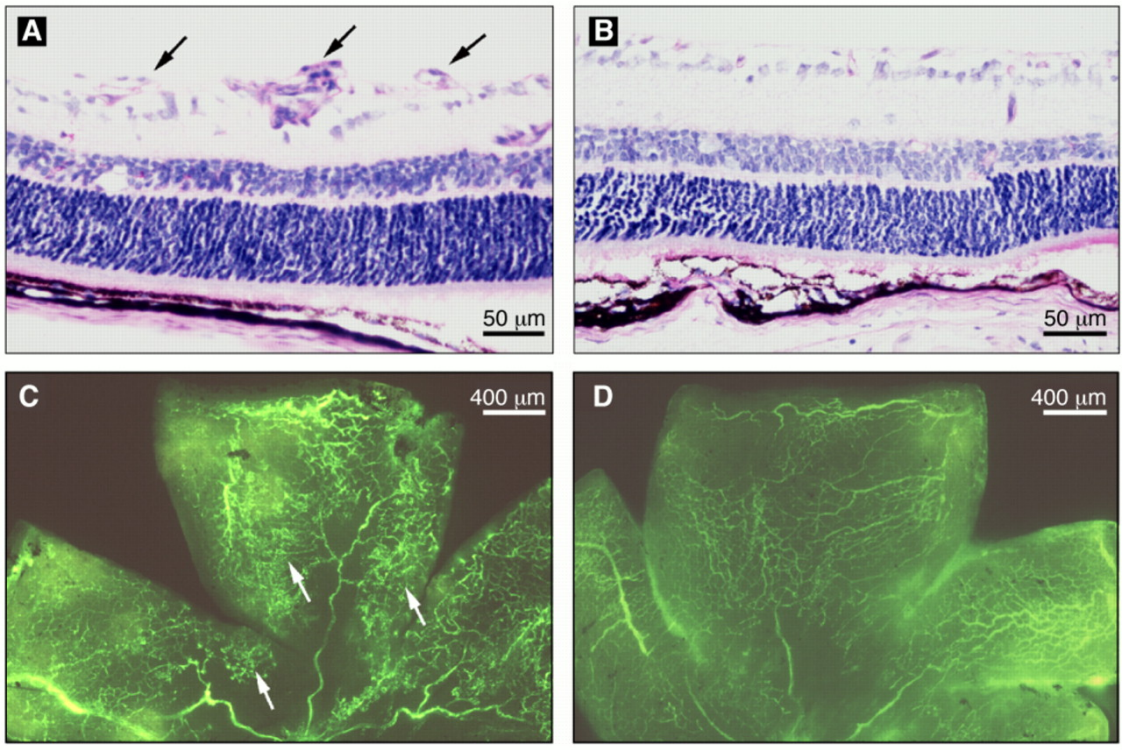

Fig. 10. Schematic representation of retinal neovascularization of an eye in nontransgenic mouse**.** Adapted from Smith et al., 1997; *Science*.

Unger et al. first proposed a hypothesis that decreases in SST secretion could be responsible for hyperinsulinemia and hyperglucagonemia in T2DM and obese individuals (Unger et al., 1977). Further studies to work on this hypothesis were hampered because of plasma immunoreactive somatostatin (IRS) which is released from the other extrapancreatic tissues. Although clinical studies on T2DM patients (Gutniak et al., 1987) (Segers et al., 1989) (Conlon et al., 1983) confirms the hypothesis of Unger, further studies needs to be done to reveal this question.

Gerich et al., have demonstrated that hyperglycemia was better controlled when SST was given along with insulin in T1DM patients compared to the administration of insulin alone (Gerich et al., 1977). The study summarized that long acting and selective SST analogue along with insulin can be useful in controlling diabetes. Later on, Grossmen et al., showed that subcutaneous administration of octreotide improved glycemic control in patients with T1DM (Grossmen et al., 1989). Williams et al., performed a short term study with subcutaneous treatment with octreotide and showed improvement in postprandial glycemic control in T2DM individuals. Although this study suggests that octreotide lacked the selectivity and is not therapeutically useful for the treatment of T2DM (Williams et al., 1988).

Octreotide had been approved by the Food and Drug Administration (FDA) for the treatment of hormone secreting tumors (Gorden et al., 1989). Octreotide was the first long-acting analogue which was used for the treatment of acromegaly or carcinoid tumors along with lanreotide and vapreotide which were introduced clinically later. Acromegaly is a syndrome, which is characterized by the presence of impaired glucose tolerance and insulin resistance (Baldelli et al., 2003). Octreotide is almost ten times more potent and have longer active ability than SST-14 to inhibit growth hormone (GH) release (Bauer et al., 1982). Treatment of acromegaly with octreotide had long lasting suppression on GH levels and showed no side-effects or rebound effects of GH secretion (Plewe et al., 1984).

Since SST inhibits both insulin and glucagon secretion, development of selective, potent and long-acting SST analogue which can selectively inhibit glucagon and GH secretion without effecting insulin secretion could be the ideal drug for the treatment of diabetes (Rivier et al., 1975) (Lins et al., 1980). Two such selective analogues of SST which can

be used for the treatment of T1DM and diabetic retinopathy due to their selective inhibition, are RC-121 and RC-160. They potently inhibits GH secretion with only weak inhibiting effect on insulin and glucagon secretion (Cai et al., 1986).

1.9. Hypothesis of the present study

Several studies have demonstrated the distribution of SSTRs in the endocrine pancreas both in rodents and humans, but the exact physiology of SSTRs in the endocrine pancreas still needs to be identified. The other method for the evaluation of SSTRs is by inactivating of gene encoding a particular receptor subtype in animal model. In rodents SSTR2 inhibits glucagon secretion *in vitro* while SSTR5 plays a key role in inhibition of insulin secretion. In our recent study Strowski et al., have demonstrated that SST-14 inhibits glucagon secretion from isolated pancreatic islets via SSTR2 (Strowski et al., 2006). In this study, SSTR2 knock out mice were used with highly selective, potent non-peptidal SSTR agonists. We further attempted to understand the mechanism by which SSTR2 regulates glucose homeostasis. To answer this question, we developed a mouse model of high fat diet induced obesity with the deletion of SSTR2 gene.

Since pancreatic islets are consist of several hormonally active cell subtypes, it is not clear from studies *in vivo* or using isolated islets whether SSTR2-selective agonist directly interacts with α-cells or the effects are mediated through interaction with other cell types of the endocrine pancreas. Although our previous studies on isolated human pancreatic islets and SSTR2 knock out mice showed that SSTR2 plays a crucial role in glucagon inhibition. Our data showed that hyperglycemia can be reduced by using SSTR2 selective agonist which could be a novel therapy to treat T2DM. In our future studies we would like to reveal the mechanism involved in regulation of glucagon secretion via SSTR2 selective agonist by using permanent cell model of glucagon secreting cells (InR1-G9).

Insulin secretion and proinsulin gene transcription of β -cells are regulated by SST. In our previous studies, we have demonstrated that SSTR2 and SSTR5 inhibits insulin secretion in isolated pancreatic islets but the mechanisms involved in regulation of these processes are not well understood. To identify the mechanism involved we used the permanent cell line of rat β –cells (INS-1).

The main objective of this study are-

1. Human pancreatic A- and B- cells express SSTR1, 2, 3 and 5. However the contribution of individual receptor subtypes in the regulation of glucagon and insulin secretion in humans is not well understood. The aim of this study is to characterize the individual role of SSTRs in the regulation of human glucagon and insulin secretion in a system of isolated human pancreatic islets.
2. Previous studies have shown that in rodents SSTR2 plays a potent role in the glucagon inhibition (Strowski et al., 2000) (Strowski et al., 2006). In T2DM hypersecretion of glucagon contributes to hyperglycemia. In this study we attempted to characterize the role of SSTR2 on glucose homeostasis in a mouse model of diet induced obesity and insulin resistance **(Fig. 11)**.

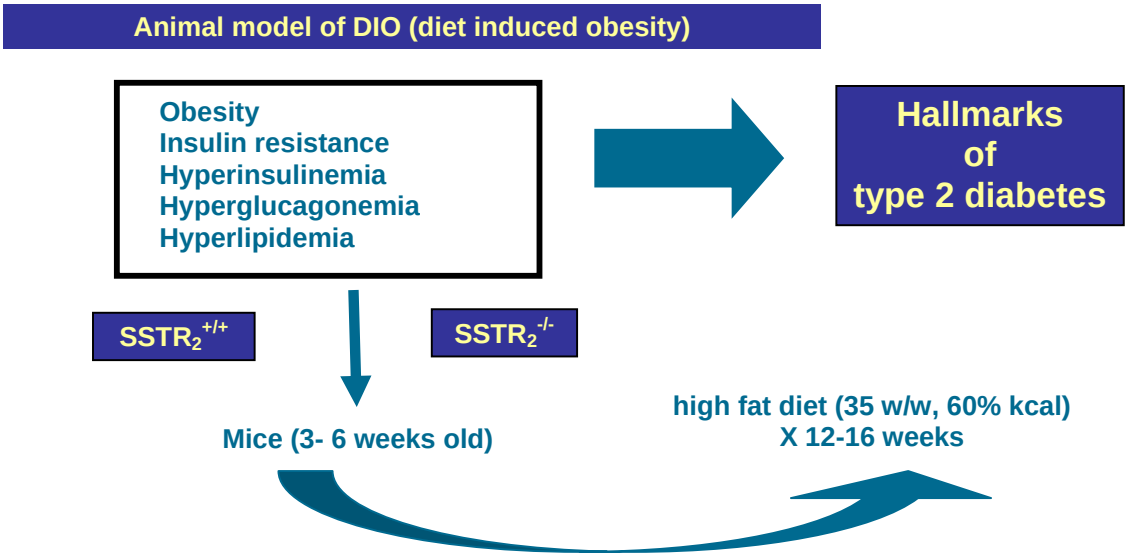

Fig. 11. Schematic representation of the function of SSTR2 in T2DM

3. Secretion of endocrine pancreatic hormones are tightly controlled by the interplay with each other. For instance SST regulates both insulin and glucagon secretion. In our previous study, we have shown that SSTR2 inhibits glucagon secretion from isolated mice pancreatic islets (Strowski et al., 2000) (Strowski et al., 2006). Since islets are comprised of several hormonally active cell subtypes, it is still a

question whether effect of SSTR2 was directly with A-cells or it was mediated by the other cells of the endocrine pancreas. In order to answer this question, we selected the permanent cell line of pancreatic A-cells (InR1-G9 hamster glucagonoma).

4. A- and B- cells of the endocrine pancreas express all SSTRs. SST inhibits insulin secretion and proinsulin gene transcription but the underlying mechanism involved in regulation of these processes is not well characterized. This study was designed to characterize the mechanisms responsible for SSTR dependent regulation of insulin secretion and proinsulin gene expression using INS-1 cells (rat insulinoma).