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Abstract

Although type 2 diabetes is a disease with complex metabolic nature, is frequently associated with dysfunctional pancreatic insulin producing β-cell. Due to their cell membrane localization, the GPCRs are easily accessible target to restore β-cell function and consequently treatment of T2D. The overall aim of the thesis was to compare the expression level of islet GPCRs in mouse and human as well as study functional impact of the stabile ATP and UTP metabolite i.e. adenosine or UDP-glucose on insulin secretion.

A comprehensive analysis of islet GPCRs demonstrated species differences concerning GPCR expression and function in human and mouse islets, (Paper I). Thus, it was found that the Adenosine A3 receptor (ADORA3), GAL1 (GALR1), GAL2 (GALR2) and GAL3 GALR3) were expressed only in mouse islets where activation of each inhibited glucose-induced insulin secretion (GSIS) from mouse islets, with no effect on human islets. Conversely, the somatostatin receptor 1 (SSTR1) was abundant only in human islets and its selective activation inhibited GSIS from human islets, with no effect on mouse islets. On the other hands, adenosine A1 receptors (A1R), was abundantly expressed in both mouse and human islets, which upon activation exerted inhibitory effect on the GSIS. Functional inhibition of A1R either by knockdown or by a specific antagonist potentiated GSIS (Paper II). While activation of uridine diphosphate (UDP) receptor i.e. P2Y6 which is expressed in both mice and human islet positively potentiated GSIS, activation of P2Y14 by UDP-glucose suppressed GSIS in both human and rodent insulin releasing β-cells. Inhibitory action of P2Y14 was mediated through a signaling cascade involving PTX-sensitive Gi protein.

In conclusion, the generated data show that there are certain GPCRs with a differential expression in human compared to mouse pancreatic islets, which likely might have an impact on the translatability of mouse studies to the human.

In view of these novel findings, it can be proposed that specific blockers of A1R or P2Y14 as well as a selective activator of P2Y6 that collectively improve the secretory capacity of pancreatic β-cells could be new potential candidates in the therapeutic strategy for T2D treatment.

Key words GPCRs, A1R, P2Y14, P2Y6

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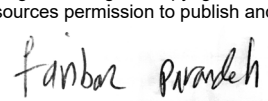
Signature  Date 2022-03-28

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Abbreviations

AC	Adenylate cyclase
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanidine monophosphate
cDNA	Complement DNA
DAG	Diacylglycerol
ER	Endoplasmic reticulum
GLP1	Glucagon-like peptide 1
GPCRs	G protein-coupled receptors
GSIS	Glucose stimulated insulin secretion
HbA1c	Glycated hemoglobin
IGT	Impaired glucose tolerance
IP3	Inositol triphosphate
MODY	Maturity-onset diabetic of the young
NO	Nitric oxide
NOS	Nitric oxide synthase
PDE	Phosphodiesterase enzyme
PIP2	Phosphatidyl inositol biphosphate
PKA	Protein kinase A
PKC	Protein kinase C
PP	Pancreatic polypeptide
siRNA	Small interfering RNA
T1D	Type 1 diabetes
T2D	Type 2 diabetes

List of Papers Included in the Thesis

1. A comparative analysis of human and mouse islet G-protein coupled receptor expression. Stefan Amisten, Patricio Atanes, Ross Hawkes, Inmaculada Ruz-Maldonado, Bo Liu, Fariborz Parandeh, Min Zhao1, Guo Cai Huang, Albert Salehi & Shanta J. Persaud. Scientific Reports (2017).

2. Absence of adenosine A1 receptors unmasks pulses of insulin release and prolongs those of glucagon and somatostatin. Albert Salehi, Fariborz Parandeh, Bertil B. Fredholm, Eva Grapengiesser, Bo Hellman. Life Sciences 85 (2009) 470–476.

3. Uridine diphosphate (UDP) stimulates insulin secretion by activation of P2Y6 receptors. Fariborz Parandeh, Sandra Meidute Abaraviciene, Stefan Amisten, David Erlinge, Albert Salehi. Biochemical and Biophysical Research Communications 370 (2008) 499–503.

4. Inhibitory effect of UDP-glucose on cAMP generation and insulin secretion. Fariborz Parandeh, Stefan Amisten, Gaurav Verma, Israa Mohammed Al-Amily, Pontus Dunér and Albert Salehi. JBC (2020)

Publications not Included in the Thesis

1. Signal Transduction in Islet Hormone Release: Interaction of Nitric Oxide with Basal and Nutrient-Induced Hormone Responses. Albert Salehi, Fariborz Parandeh and Ingmar Lundquist. Cell. Signal. Vol. 10, No. 9, pp. 645–651, 1998

2. The Nitric Oxide Synthase Inhibitor NG-nitro-L-Arginine Methyl Ester Potentiates Insulin Secretion Stimulated by Glucose and L-Arginine Independently of its Action on ATP-Sensitive K+ Channels. Albert Salehi, Fariborz Parandeh and Ingmar Lundquist. March 1998 Bioscience Reports 18(1):19-28

Introduction

Blood sugar regulation is the process by which the levels of blood sugar, primarily glucose is maintained by the body within a narrow range. This phenomenon of tight regulation is commonly referred to as **glucose homeostasis**. Although insulin and glucagon are the most well-known hormones involved in the blood glucose regulation, there are still other hormones that might affect blood glucose indirectly such as stress hormones adrenaline and cortisone known to negatively affect glucose uptake by peripheral insulin-targeted tissues (DeFronzo RA et al, 2015). Sympathetic/parasympathetic nervous system are also involved in the blood glucose regulation by affecting endocrine cells of pancreatic islets (Revathy Carnagarin et al, 2018). Blood glucose regulation is very important to the maintenance of the normal body homeostasis in mammals. The brain does not have any energy storage of its own and as such needs a constant flow of glucose. Thus, both hypoglycemia and hyperglycemia negatively affect the functionality of brain tissue (Ashish K Rehni 2015). Both long lasting hypoglycemia and hyperglycemia are associated with brain damage (Ashish K Rehni 2015).

Diabetes mellitus (DM), commonly referred to as **diabetes** is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both, Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycemia. Symptoms of high blood sugar include frequent urination, increased thirst, and increased hunger. If left untreated, diabetes can cause many complications, Acute; life-threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or the non-ketotic hyperosmolar syndrome. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Diabetes is roughly divided into two groups. Type 1 diabetes and Type 2 diabetes. Although there seems to be a dispute about another subdivision of diseases in more defined grouping.

Classification of diabetes mellitus

Type 1 *diabetes mellitus* (β-cell demise, usually leading to absolute insulin deficiency)

This form of diabetes i.e. type 1 diabetes mellitus (T1D), which accounts for only 5–10% of patients with diabetes, previously encompassed by the terms insulin-dependent- or juvenile-onset diabetes mellitus (IDDM), results from a cellular-mediated autoimmune destruction of the pancreatic β-cells. Markers of the immune destruction of the β-cell include islet cell autoantibodies, autoantibodies to insulin, autoantibodies to glutamic acid decarboxylase (GAD), and autoantibodies to the tyrosine phosphatases IA-2 and IA-2β (Krischer JP et al,2019). Although the mechanisms behind T1D development is not fully understood, autoimmune destruction of β-cells has been considered that further might have multiple genetic predispositions and environmental factors been involved. However, the interplay between these factors is poorly understood.

Type 2 *diabetes mellitus* (β-cells poorly respond to carbohydrate challenge)

This form of diabetes is a global disease caused by the inability of pancreatic β-cells to secrete adequate insulin in response to carbohydrate (DeFronzo RA et al,2015). Type 1 diabetes mellitus (T2D) previously referred to as non-insulin-dependent diabetes (NIDDM) or adult-onset diabetes, encompasses individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency at least initially. There are probably many different causes of this form of diabetes, Although the specific etiologies are not known, autoimmune destruction of β-cells seems not to be involved (Ahlqvist E et al,2018). It is often associated with a strong genetic predisposition (Ahlqvist E et al,2018). However, the genetics of this form of diabetes are complex and not clearly defined. This form of diabetes frequently goes undiagnosed for many years because the hyperglycemia develops gradually and at earlier stages is often not severe enough for the patient to notice any of the classic symptoms of diabetes. Since T2D is characterized by a reduced β-cell response to glucose, knowledge regarding the signaling molecules capable of modulating insulin and glucagon secretion are of particular interest for the treatment of T2D. There are, however, a number of subgroups within T2D as has been reported (Ref) but a certain categories are of interest to mention that could also regarded as own or new groups such as gestational diabetes and maturity-onset diabetes of the young (MODY).

Gestational diabetes

The third main form and occurs when pregnant women without a previous history of diabetes develop high blood sugar levels during pregnancy where reportedly in addition to genetic/epigenetic factors, elevated pregnancy hormones are also involved. Gestational diabetes normally occurs in 2^{nd} or 3^{rd} trimester of pregnancy (Moon JH, et al,2017).

Genetic defects of the β-cell.

Several forms of diabetes are associated with monogenetic defects in β-cell function. These forms of diabetes are frequently characterized by onset of hyperglycemia at an early age (generally before age 25 years) (Ellard SC et al, 2008). They are referred to as maturity-onset diabetes of the young (MODY) and are characterized by impaired insulin secretion with minimal or no defects in insulin action. They are inherited in an autosomal dominant pattern.

G protein–coupled receptors (GPCRS)

G protein coupled receptors (GPCRs) constitute a large protein family of receptors that sense molecules outside the cell and activate inside signal transductions pathways and, ultimately, cellular responses (Robas N, et al 2003). As it has been reported GPCRs constitute the largest group of cell surface receptors in man, and are also the targets of ~35% of all prescription medicines (Flower DR.1999). For example, Glucagon-like peptide-1 receptor (GLP-1R) is expressed by both human and rodent β-cells and GLP-1 is well studied hormone that play a crucial role in islet function by regulating insulin secretion, β-cell proliferation and survival via activation of GLP-1R (Buteau J, et al2003). A great numbers of islet GPCRs are still orphan GPCRs for which no known endogenous ligands have been identified, and these receptors constitute a large untapped pool of potential novel drug targets.

After binding of a ligand to a GPCR a conformational change would occur. After that the receptor functions as a guanine nucleotide exchange factor. G protein releases GDP and binds GTP. G protein has 3 subunits (α, β and γ) and α subunit has four type ($G_{\alpha s}$, $G_{\alpha i/o}$, $G_{\alpha q/11}$, $G_{\alpha 12/13}$) (Flower DR, 1999; Buteau J, et al 2003). Depending on the bound subunit complex i.e. G_s or G_i either stimulation or inhibition with the different intracellular signaling pathways will occur. Normally, the GPCRs affects to main and different cellular pathways cAMP/PKA or Phospholipase C (PLC) and diacylglycerol (DAG) generating IP3 further affecting cellular [Ca2+]i (Seino S & Shibasaki T, Physiol Rev 85,2005).

The cAMP signaling pathway starts with activation of membrane bound adenylate cyclase (AC) and increase the cAMP that further activates protein kinase A (PKA) with following phosphorylating cascade of a number of up-stream proteins which ultimately play a role in intracellular $[Ca^{2+}]_i$ oscillation and insulin release (Seino S & Shibasaki T, Physiol Rev 85,2005).

The phosphatidylinositol signal pathway begins whit activation of the membrane bound enzyme phospholipase C cut the phosphatidyl inositol biphosphate to membrane bound diacylglycerol and diffusible inositol triphosphate (IP3) (Liang Y & Matschinsky FM,1994). IP3 is a signal substance for receptor on the endoplasmic reticulum for transient of calcium which is important for calcium $[Ca^{2+}]_i$ oscillation and pulsatile insulin release. diacylglycerol (DAG) activate protein kinase C (PKC). The effects of PKC and PKA are not additive, suggesting that activation of either one way converge on the same secretory pathway in the regulation of insulin secretion (Liang Y & Matschinsky FM,1994).

It is well-established that secretion of hormones from islets of Langerhans is regulated by activation of islet cell GPCRs by neurotransmitters, paracrine actions of islet hormones and by circulation hormones (Amisten et al,2013). Parasympathetic and sympathetic neurotransmitters act at specific muscarinic and adrenergic GPCR subtypes to potentiate and inhibit the stimulatory effects of nutrients on insulin secretion, to allow finetuning of the insulin secretory response (Ahren et al.,2000).

In addition, glucagon stimulates insulin and somatostatin release, while somatostatin inhibits glucagon and insulin release (Jones PM PS, Textbook of Diabetes 2010, pp87-103). Furthermore, GLP-1, an incretin released from the gastrointestinal tract following food intake, acts at GPCRs on islet β- and α-cells to stimulate insulin and inhibit glucagon secretion (De Marinis et al.,2010), and GIP, another incretin, also potentiates glucose-induced insulin release. The GLP-1 receptor is probably the most well characterized of all islet GPCRs, and several GLP-1 receptor agonists and DPP4 inhibitor drugs that stabilize incretin levels are in widespread clinical use as therapies for type 2 diabetes (T2D) (Tuch et al.,2016). A number of other GPCRs, including GPR119, FFAR1, GPRC5B and GPRC5C, all of which are expressed by human islets (Amisten etal,2013), have also emerged as drug target candidates for the treatment of T2D (Oh Da et al.,2016;Soni et al.,2013)).

Human islets express almost 300 additional GPCRs (Amisten et al,2013; Regard et el.,2008; Regard et el.,2007), but most of these have poorly characterized roles in islet physiology (Amisten et al,2013). Due to the limited availability of human islets, the vast majority of all physiological and pharmacological studies on the regulation of islet hormone secretion have been carried out using isolated mouse islets. There was a need to evaluate the similarity of GPCR expression between human islets, the primary therapeutic target tissue, and mouse islets, the primary model system tissue. In our recent study human islet GPCR mRNA profiles have been compared with

those of islets isolated from mouse and a core set of 121 GPCR mRNAs were found to be expressed by islets of both human and mouse. A1R, P2Y6- and P2Y14 receptors are among these receptors.

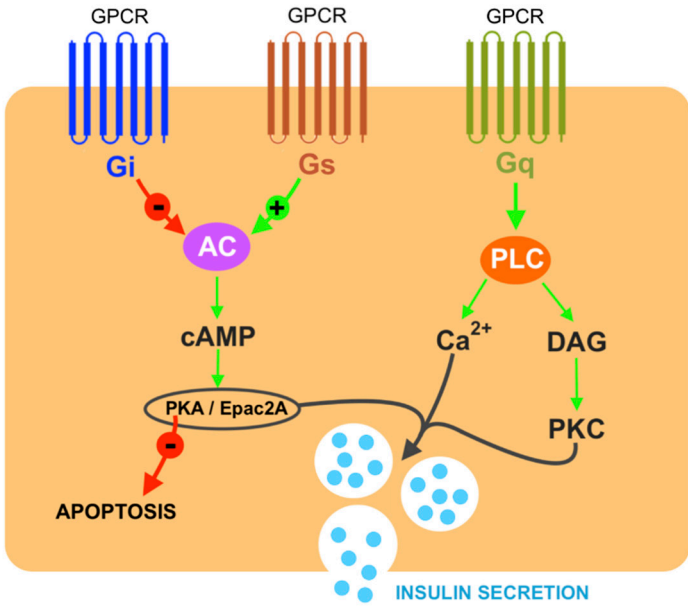

Figure 1. A schematic illustration of GPCR signaling pathway in β-cells showing that GPCRs can influence insulin secretion by the specific receptors coupled to G_s (potentiation of insulin secretion), G_i (inhibition of insulin secretion) or G_q/G_{11} (potentiation of insulin secretion).

Considering the fact that GPCRs are targeted by almost 40% of the current drugs on the market and particularly for being easily accessible targets that makes GPCRs a great pharmaceutical interest. However, most of GPCRs expressed by pancreatic islets are still orphan, without any well-known ligands, which require an extensive research to explore their therapeutic potentials (Amisten et al 2013). We were interested in investigating the following de-orphanized receptors; A1R, P2Y2, P2Y4, P2Y6 as well as P2Y14 to study their impact on the β-cells function. All of these GPCRs are expressed by almost all cells in the body and they are target for the extracellular adenine and uracil nucleotides (Burnstock G 2006).

In general, purinoceptors are a family of plasma membrane molecules that are found in almost all mammalian tissues. They are divided into P1R and P2R. P1R is a GPCR which response to adenosine. P2 receptors have further been divided into subclasses: P2X, P2Y. P2X receptors are ligand-gated ion channels which desensitized quickly.

P2Y receptors are G protein coupled receptors which are responsive to purine and pyrimidine nucleotides and nucleotide sugars (Ralevic & Burnstock, 1998; Abbracchio et al., 2006)

P2Y receptors can be divided on the basis of their endogenous ligands into adenine nucleotide-preferring (P2Y1, P2Y11, P2Y12 and P2Y13 receptors) and uracil nucleotide or UDP-sugar-preferring (P2Y2, P2Y4, P2Y6 and P2Y14 receptors) (von Kugelgen,2006). Alternatively, P2Y receptors can be distinguished as P2Y1-like family and P2Y12-like family based on their sequence alignments and effector coupling. The P2Y1-like family couples to Gq protein and involves an activation of the phospholipase C (PLC) signaling pathway (Costanzi et al., 2004). This sub-family contains P2Y1, P2Y2, P2Y4, P2Y6 and P2Y11, although P2Y11 receptor can couple to Gs protein too, leading to an activation of adenylyl cyclase (Communi et al., 1997). The P2Y12-like family can couple to Gi protein leading to an inhibition of adenylyl cyclase (Jacobson et al., 2012). The sequence homology between the two sub-families is low, for instance, the sequence identity between P2Y1 and P2Y12 receptors is only 20%. While the sequence identity between the members within the same sub-family is higher, for instance, the sequence identity between P2Y12 and P2Y14 receptors is 45% (Jacobson et al., 2010). P2Y receptors have a wide distribution throughout the body and they mediate various responses in a variety of tissues (see reviews by Burnstock, 2007; Burnstock et al., 2010).

Molecular mechanism for purinoceptors A1R, P2Y6 and P2Y14

Insulin secretory granules contain ATP, ADP, UTP, UDP. ATP is very rapidly hydrolyzed to adenosine by ecto-nucleotidases. UDP-glucose is a component of glycosylation reactions that take place intracellularly in many cell types especially in hepatocytes, in the process of glycogen metabolism.

P1 receptors (Adenosine receptors)

Adenosine is a purine nucleoside composed of a molecule of adenine attached to a ribose sugar molecule. Adenosine plays an important role in biochemical processes. Adenosine is an endogenous purine nucleoside that modulates many physiological processes. Cellular signaling by adenosine occurs through adenosine receptor. All adenosine receptors (P1 receptors) can be sub-divided into four distinct subtypes. (A_1, A_{2A}, A_{2B}, and A_3). (Olah & Stiles, 2000; Fredholm et al., 2001). All adenosine receptors subtypes are G-protein-coupled receptors. The four receptor subtypes are further classified based on their ability to either stimulate or inhibit adenylyl cyclase.

A1 and A3 are negatively coupled to adenylyl cyclase through Gi/o protein, A2A and A2B receptors are positively coupled to adenylyl cyclase through Gs protein (Reshkin et al., 2000).

P2Y6 Receptor

P2Y6 receptor has UDP as ligand. The receptor is a GPCR (Gq) which activate the enzyme phospholipase C which cleaves phosphatidyl inositol biphosphate (PIP2) to the membrane bound diacylglycerol (DAG) and diffusible inositol triphosphate (IP3). IP3 acts on receptor on endoplasmic reticulum to release calcium. Induction of short-lived transients of $[Ca^{2+}]_i$, which temporarily interrupt the voltage-dependent entry of Ca^{2+} by activating a hyperpolarizing K^+ current (Grapengiesser et al. 2003). The calcium transients are supposed to regulate the calcium oscillations and resulting pulsatile insulin release from pancreatic β-cells. DAG in the inner membrane surface activates PKC.

P2Y14 Receptor.

The P2Y14 receptor (also known as GPR105) is the most recently identified member of the P2Y family of receptors for adenine and uridine nucleotides and nucleotide sugars and is responsive to uridine-5'-diphosphate-glucose (UDP-glucose) and other sugar nucleotides (Chambers et al., 2000; Abbracchio et al., 2003). P2Y14 receptor is activated by UDP-glucose and other nucleotide sugars, with a rank order of the potency of P2Y14 receptor ligands as follows: UDP-glucose ≥ UDP-glucuronic acid > UDP-galactose > UDP-N-acetylglucosamine (Chambers et al., 2000; Ko et al., 2007). MRS2690 (2-thiouridine-5′-diphosphoglucose) has 7-fold greater potency than UDP-glucose at P2Y14 receptors (Ko et al., 2009). UDP-glucose is a potent agonist at P2Y14 receptor (Carter et al., 2009).

The human P2Y14 receptor shares 45% amino acid identity with human P2Y12 and P2Y13 receptors and 22% with the P2Y1 receptor (Abbracchio et al., 2003; Moore et al., 2003). In our recent study, we found that Dose dependent activation of P2Y14 by UDP-G suppressed glucose stimulated insulin secretion (GSIS) and knockdown of P2Y14 abolished the UDP-G effect.

Aims

The general aim of this thesis was, on one hand, to identify similarities and differences in GPCR expression in human and mouse islets and on the other hand, to investigate the role of three selected GPCRs, which are express in both human and rodent islets on the hormone secretion. The study was performed on isolated pancreatic islets and on the β-cell cell line INS-1 832/13 cells.

Paper I

The aim of paper I was to understand which GPCRs are present on human islet, and if mouse islet shows a similar expressional pattern and can be used as a translational model system for the GPCR of interest. The created atlas over common GPCRs between human and mouse pancreatic islets are essential for development of new diabetes therapeutics.

Paper II

The aim of paper II was to examine whether adenosine via A1 receptors (A1R) interferes with pulsatile islet hormone release and compare if the insulin pulses are synchronous or antisynchronous with glucagon and somatostatin pulses.

Paper III

The aim of paper III was to examine the transcriptional pattern of the pyrimidine P2Y receptors i.e. P2Y2, P2Y4, and P2Y6 compared to P2Y1 in mouse pancreatic islets. We also wanted to evaluate the possible effect of these receptors on the insulin and glucagon secretion.

Paper IV

The aim of paper IV was to study the effect of UDP-glucose on β-cell function in relation to P2Y14 expression and also evaluate the role of P2Y14 as possible drug candidate.

Materials and methods

A brief description of the experimental procedures and analytical techniques is given below. A more detailed description of different methods during studies as well as the source of chemicals and materials can be found in each separate paper.

Isolated mouse islets

Male or female mice (c57BL/6 strains) were purchased from Charles River, Harlan Janvier Laboratory (Paris), weighing 25–30 g were used in our study. They were given a standard pellet diet with tap water ad libitum. Pancreatic islets were isolated by collagenase digestion of the exocrine pancreas (Isra Mohammad Al-Amily et al 2019). Local ethical committee had approved the use of animals in our studies.

Isolated human islets

Isolated human pancreatic islets from cadaveric organ donors (Prodo, USA) with 90 % purity had been cultured in CMRL 1066 medium for around 5 days prior to use. The islets were then hand-picked under stereomicroscope at room temperature and subjected to different treatment as indicated in the relevant papers. Local ethical committees approved the use of isolated human islets in our experiments.

INS-1 832/13 cells

The Rat glucose-responding insulinoma cell line INS-1 832/13 was kindly provided by Dr. Chrisopher B. Newgard; Duke University, School of Medicine (Hohmeier, H. E., and Newgard, 2004). The cells were seeded (350 000 cells/well) in a 24-well plate with 1 ml/well complete RPMI 1640 medium supplemented with 11.1 mM D-glucose and 10% FBS, 2% INS-1 supplement (18), 5 ml penicillin/streptomy-cin (10,000 units/10 mg/ml), and 10 mM Hepes (HyClone, Logan, UT, USA). The cells were cultured in a humidified atmosphere with 5% CO2 at 37°C for 24 h (Mohammad Al-Amily et al 2019). When the cells reached an appropriate confluence for the experiments, they were washed with PBS and subjected to the different experimental procedures as indicated in the papers.

Biochemical and radio-immunological analysis

Hormone analysis

The released hormones in perfusion medium or in the incubation medium were analyzed by RIA (Salehi et al Am j physiology 1996) or ELISA (Mohammad Al-Amily et al 2019).

cAMP detection

For the measurement of cAMP, INS-1 832/13 cells were incubated for 60 min at 1 or 16.7 mmoI/1 glucose in the presence or absence of the test agent. The incubation buffer buffer also contained 3-isobutyl-1-methylxanthine (IBMX) (100 mM) to prevent the hydrolysis of cAMP by cellular phosphodiesterase (Muhammed SJ et al,2012). After incubation, the cells were washed with PBS and stored in RIPA buffer containing, HCl (100 mM) and IBMX (100 mM) for subsequent analysis of cAMP, which was measured using a direct cAMP ELISA kit (AD-900-066) (Enzo Life Sciences) according to the manufacturer's instructions. The protein concentrations in the cell lysates were measured by a BCA kit (Nr 23225; Thermo Fisher Scientific).

In addition to the above-mentioned methods, there were also specific technique or analysis of material used in each paper as follow:

Study I

In this study isolated mouse and human islets from non-diabetic organ donors were used. for extraction of RNA a modified TRIzol protocol was used. GPCR expression was quantified relative to the house keeping gene GAPDH by quantitative real-time PCR (qPCR).

For analysis of insulin secretion groups of 3 or 12 isolated mouse or human islets were incubated for 1 hour in a physiological salt solution (Get Go GM 1936) in the absence or presence of the indicated agents. The secreted insulin was quantified by radioimmunoassay (Jones et al.,1988).

Study II

The impact of A1 receptor on insulin secretory response of pancreatic β-cells in relation to glucagon and somatostatin secretion from α and δ cells were studied in a pancreas perfusion model. Pancreas was perfused in mice expressing or lacking the A1 receptor and the released hormones were measured with radioimmunoassay. Cytoplasmic (intracellular) Ca^{2+} $[Ca^{2+}]_i$ transients was recorded using fura-2 indicator in isolated β-cells from the splenic part of the pancreas since the islets from

this region contain >90% β-cells, which have a normal secretory response to glucose (Hahn et al. 1974).

Study III

Isolated islets were either dissolved immediately in TRIzol (Invitrogen) and stored at -80 ºC for RNA purification or subjected for β-cell purification by repeated counter-flow elutriation using first a standard chamber and then a Sanderson chamber Beckman (Palo Alto, CA) as previously applied for ECL-purification (E. Lindström.at.al,1997) with some modifications. This cell preparation, (~80% β-cells) was then subjected to density gradient centrifugation. The purity of each β-cell preparation was assessed by RIA measurement of insulin, glucagon, Somatostatin and PP per mg protein (S.S Qader.at.al 2007). The final cell preparations, consisting of around 95% β-cells, were then collected in TRIzol (Invitrogen) and stored in –80 ºC. All quantitative real-time PCR (qPCR) primers were designed using Vector NTI software (Invitrogen, Informax, UK). Relative gene expression levels were determined as described elsewhere (Pfaffl M.W. et al.,2001).

Study IV

Confocal microscopy

Handpicked islets were washed twice and fixed with 3% paraformaldehyde for 10 min, followed by permeabilization with 0.1% Triton X-100 for 15 min. Insulin staining was carried out using a primary guinea pig anti-insulin antibody (1:300) followed by incubation with fluorescent- conjugated secondary antibodies (1:100). P2Y14 protein expression in insulin-positive cells in human and mouse islets as well as INS-1 cells was determined by confocal microscopy using the Zen 2009 (Carl Zeiss, Oberkochen, Germany) software and rabbit polyclonal anti-GPR105 (P2Y14) antibodies at a 1:200 dilution. Fluorescence was visualized with a Zeiss LSM510 confocal microscope by colocalization analysis of islet P2Y14 with insulin (indicator of β-cells) in islets was performed using the ZEN2009 software based on Pearson's coefficient analysis, which recognizes the colocalized pair by comparison pixel by pixel intensity (Zhang at al 2019; Al-Amily,at.al 2019; Costes at al 2004). The plasma membrane/cytosol ratio was calculated by mean intensity of plasma membrane to mean intensity in cytosol, as described previously (Zhang at al 2019; Al-Amily,at.al 2019; Costes at al 2004).

P2Y14 SiRNA

Transient knockdown of P2Y14 in INS-1 832/13 cells were performed by siRNA transfection (36-42h). After transfection, the media was replaced with complete

RPMI 1640 media with antibiotics and the INS-1 832/13 cells were cultured for additionaly 4-6h for recovery before being subjected to different experimental protocol.

Western blot

For the visualization of the P2Y14 protein by Western blots, INS1 832/13 lysates representing 30 µg of total protein were run on SDS-polyacrylamide gels (7.5%9 (Bio-Rad, Hercules, CA, USA). After electrophoresis, proteins were transferred to nitrocellulose membranes (Bio-Rad, Hercules, CA, USA). The membranes were blocked in LS-buffer (10 mM Tris, pH 7.4, 100 mM NaCl, 0.1% Tween-20) containing 5% non-fat dry milk powder for 40 min at 37°C. Subsequently the membranes were incubated over night with the following primary antibodies: polyclonal rabbit anti-GPR105 (P2Y14) antibody (1:150) and polyclonal rabbit anti-tubulin antibody (1:150), at room temperature. After washing (three times) in LS-buffer the membranes were finally incubated with a horseradish peroxidase-conjugated and anti-rabbit antibodies (1:500). Immunoreactivity was detected using an enhanced chemiluminescence reaction (Pierce, Rockford, IL, USA). The results were quantified by densitometric analysis using the Bio-Rad software.

Cell viability and apoptosis

Cell viability (measuring the reductive capacity of cells) was analyzed by MTS and apoptosis was measured with the Cell Death Kit (Roche Diagnostics), which quantifies the appearance of cytosolic nucleosomes in both cultured human islet homogenates and cultured INS-1 832/13 homogenates as reported previously (Zhang at al 2019). Cell proliferation by counting INS-1 832/13 cells using a Bürcker chamber as described previously (Soni at al 2013).

Results and discussion

Paper I

A comparative analysis of human and mouse islet G-protein coupled receptor expression

G-protein coupled receptors (GPCRs) are essential for islet function, but most studies use rodent islets due to limited human islet availability.

We have systematically compared the GPCR mRNA expression in human and mouse islets to determine to what extent mouse islets can be used as surrogates for human islets to study islet GPCR function, and we have identified species-specific expression of several GPCRs. The A3 receptor (ADORA3) was expressed only in mouse islets (Fig. 2) and the A3 agonist MRS 5698 inhibited glucose-induced insulin secretion from mouse islets, with no effect on human islets. Similarly, mRNAs encoding the galanin receptors GAL1 (GALR1), GAL2 (GALR2) and GAL3 GALR3) were abundantly expressed in mouse islets but present only at low levels in human islets (Fig. 3), so galanin inhibited insulin secretion only from mouse islets. Conversely, the sst1 receptor (SSTR1) was abundant only in human islets (Fig. 4) and its selective activation by CH 275 inhibited insulin secretion from human islets, with no effect on mouse islets. Our comprehensive human and mouse islet GPCR atlas has demonstrated that species differences do exist in islet GPCR expression and function, which are likely to impact on the translatability of mouse studies to the human context.

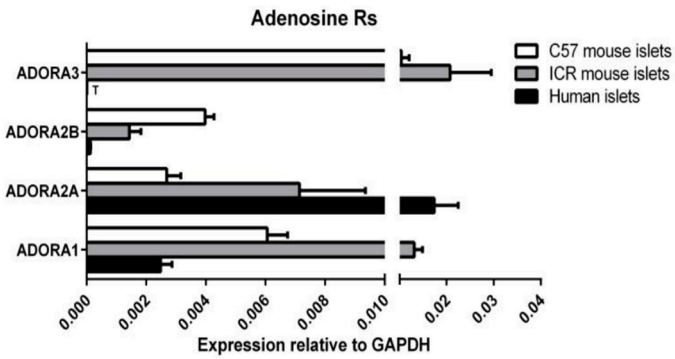

Figure 2. Expression of ADORA1, ADORA2A, ADORA2B and ADORA3 relative to GAPDH of in mouse and human pancreatic islets. Mean ± SEM for n=4 (ICR and C57 mouse islets) and n=3-4 human islet donors in each group.

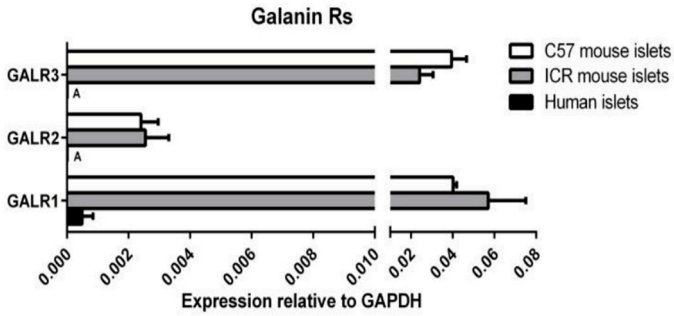

Figure 3. Expression of GALR1, GALR2, GALR3 relative to GAPDH in mouse and human pancreatic islets. Mean ± SEM for n=4 (ICR and C57 mouse islets) and n=3-4 human islet donors in each group.

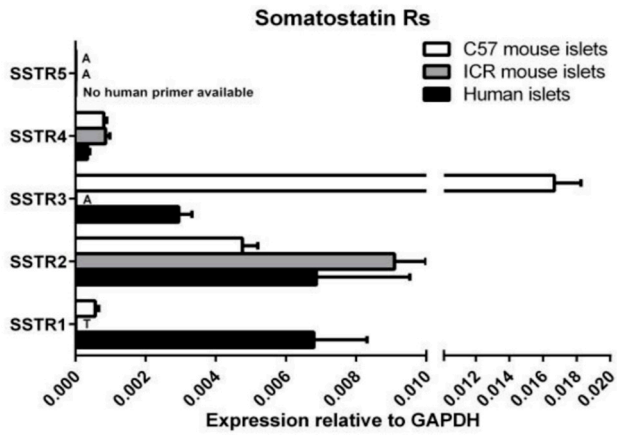

Figure 4. Expression of SSTR1, SSTR2, SSTR3, SSTR4 and SSTR5 relative to GAPDH in mouse and human pancreatic islets. Mean ± SEM for n=4 (ICR and C57 mouse islets) and n=3-4 human islet donors in each group.

Conclusion:

- Our comprehensive GPCR atlas shows that there are similarities and species differences in GPCR expression of mouse and human islets.
- The species differences in GPCR expression in the islets are likely to affect the translatability of mouse studies into the human context

Paper II

Absence of adenosine A1 receptors unmasks pulses of insulin release and prolongs those of glucagon and somatostatin

Our data showed that in addition to insulin secretion, glucose-induced glucagon and somatostatin release showed a two-phase pattern. Increase in glucose concentration was associated with an increase in Ca^{2+} transient in the β-cells. Addition of 10 μmol adenosine removed the Ca^{2+} transients supposed to coordinate the insulin release pulses. This effect of adenosine was counteracted by 100 nm of the A (1)R antagonist DPCPX. In situ perfusion of the pancreas indicated two phases of islet hormone release when glucose was raised from 3.3 to 16.7 mm. The first phase was characterized by a brief dip followed by a peak, which was more pronounced for insulin and somatostatin than for glucagon. The second phase was markedly affected by knockout of A1R. The wild-type A1R (+/+) mice, usually lacked statistically verified insulin pulses but generated anti synchronous glucagon and somatostatin pulses with half-widths of 4 min. In the A1R (-/-) mice time-average release of insulin during the second phase was almost three times higher than in the controls and 30% of the hormone was released as distinct pulses with half-widths of 3 min. The absence of the A1R receptor resulted in 50% prolongation of the pulse cycles of glucagon and somatostatin and loss of their anti-synchronous relationship. The A (1)R receptor is important both for the amplitude (insulin) and duration (glucagon and somatostatin) of islet hormone pulses. The inhibitory action of adenosine on glucose-stimulated insulin secretion seems, at least in part, be mediated by the removal of cytoplasmic Ca^{2+} transient in the β-cells.

Conclusion:

- A (1)R antagonists warrants to be investigated in more detail as an alternative to the current antidiabetic drugs for T2D.

Paper III

Uridine diphosphate (UDP) stimulates insulin secretion by activation of P2Y6 receptors

We examined the transcriptional expression and functional effects of receptors for the extracellular pyrimidine uridine triphosphate (UTP) and uridine diphosphate (UDP), on insulin and glucagon secretion in isolated mouse pancreatic islets and purified beta-cells. Using real-time PCR, the UDP receptor P2Y6 was found to be highly expressed in both whole islets and β-cells purified by repeated counter-flow elutriation, whereas no mRNA expression for UTP receptors P2Y4 and P2Y2 could be detected.

Functional in vitro experiments revealed that the P2Y6 agonist UDPβs dose-dependently enhanced insulin and glucagon release during short-term incubation (1h), while P2Y6 activation during a longer period (24h), selectively increased insulin release, especially at high glucose levels. The corresponding EC (50) value for UDPβs ranged from 3.2 x 10(-8) M to 1.6 x 10(-8) M for both glucose concentrations. The P2Y6 antagonist MRS2578 inhibited the effects of UDPβs, supporting a P2Y (6) specific effect. In addition to negative RT-PCR results, the lack of response to UTPγs a selective P2Y2/4 agonist further rule out the involvement of P2Y (2/4) receptors in the islet hormone release. Our results suggest a modulatory role for UDP via a functional active P2Y6 receptor in the regulation of islet hormone release.

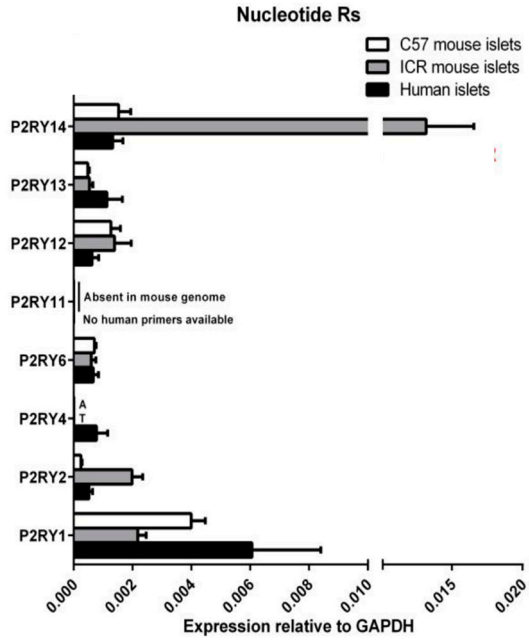

Figure 5. P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12, P2Y13 and P2Y14 relative to GAPDH in mouse and human pancreatic islets. Mean ± SEM for n=4 (ICR and C57 mouse islets) and n=3-4 human islet donors in each group.

Conclusion:

- P2Y6 is expressed in both human and rodent pancreatic β-cells.
- P2Y6 could be an attractive target for the development of new drugs potentiating GSIS.

Paper IV

Inhibitory effect of UDP-glucose on cAMP generation and insulin secretion

As mentioned earlier T2D is a global disease, caused by the inability of pancreatic β-cells to secrete adequate insulin. However, the molecular mechanisms underlying the failure of β-cells to respond to glucose in T2D remains very complex. Here, we investigated the relative contribution of UDP-glucose (UDP-G), a P2Y14-specific agonist, in the regulation of insulin release using human isolated pancreatic islets and INS-1 cells. We found that P2Y14 was expressed in both human and rodent pancreatic β-cells. Dose-dependent activation of P2Y14 by UDP-G suppressed glucose-stimulated insulin secretion (GSIS) and knockdown of P2Y14 abolished the UDP-G effect. 12-h pretreatment of human islets with pertussis-toxin (PTX) improved GSIS and prevented the inhibitory effect of UDP-G on GSIS. UDP-G on GSIS suppression was associated with suppression of cAMP in INS-1 cells. UDP-G decreased the reductive capacity of nondiabetic human islets cultured at 5 mm glucose for 72 h and exacerbated the negative effect of 20 mm glucose on the cell viability during culture period. T2D donor islets displayed a lower reductive capacity when cultured at 5 mm glucose for 72 h that was further decreased in the presence of 20 mm glucose and UDP-G. Presence of a nonmetabolizable cAMP analog during culture period counteracted the effect of glucose and UDP-G. Islet cultures at 20 mm glucose increased apoptosis, which was further amplified when UDP-G was present. UDP-G modulated glucose-induced proliferation of INS-1 cells. The data provide intriguing evidence for P2Y14 and UDP-G's role in the regulation of pancreatic β-cell function.

Conclusion:

- The receptor P2Y14 is expressed in both human and rodent β-cells.
- UDP-G has a suppressive effect on the GSIS, which is mediated via activation of P2Y14.
- P2Y14 activation by UDP-G reduces the cAMP content in the β-cells.

Summary

In summary, as presented in Figure 6 the results of present thesis indicate that P2Y14 like A(1)R is Gi protein coupled receptor, the activation of which causes a reduced AC activity that consequently leads to a decreased cellular cAMP level. cAMP plays an important role in insulin secretory response of β-cell. The mechanism of P2Y6 activation however, differ from both P2Y14 and A(1)R.

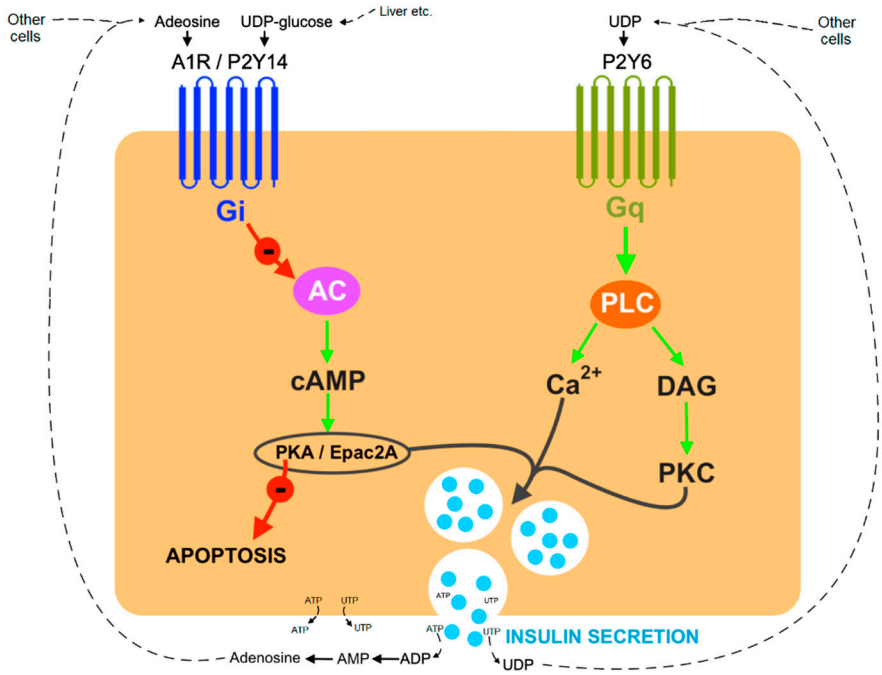

Figure 6. A schematic illustration for P2Y14 signaling pathway in β-cells showing great similarity with A(1)R activation i.e. being a Gi coupled receptor while differ from P2Y6 activation which is known to be Gq/G11 coupled receptor (see introduction).

Final Remarks

The major interpretational conclusion from the current thesis are that in spite of the complexity of T2D, there are still several ways to either prevent the metabolic disorders resulting in the β-cell dysfunction or postpone the progression of β-cell failure that results in the overt T2D. Normally a drug is developed by testing it on the rodent. The finding in the current theses reveals that there are both similarities and species differences in GPCR expression in mouse and human islets. GPCRs are easily accessible target for the drug development. Thus, our finding of species differences in GPCR expression in the islets are likely to affect the translatability of mouse studies into the human context.

Keeping in mind that the β-cell dysfunction in T2D might have different origins, we also show new targets for the restoration of β-cell dysfunction and potentiation of GSIS. Among such targets that have been studied in the present thesis are the A(1)R, P2Y6 and P2Y14 that are expressed in both human and rodent β-cells, where modulation of receptor activity was associated with the improve β-cell function.

Future perspective

It well-known that disturbed pancreatic β-cell function is the main defect finally leading to sustained hyperglycaemia and even abnormalities of intermediary metabolism that subsequently lead to progression into T2D. As the disease progresses, the β-cell ability to sufficiently respond to carbohydrate challenges and secrete adequate amounts of insulin to face hyperglycaemias declines. This will lead to additional harm on the β-cells exerted by hyperglycaemia.

GPCRs are the target for about 40-50% of the current drugs on the market. Particularly those GPCRs with known endogenous ligands could be great pharmaceutical interest for the treatment of T2D. Although most GPCRs are still orphans, we show that the de-orphanized GPCRs could also be investigated in more detail in vitro and in vivo for possible treatment of T2D. It would be of great interest to explore the impact of a more selective P2Y6 agonist as well as a more selective A(1)R and P2Y14 antagonists in the *in vivo* studies in mice.

Populärvetenskaplig sammanfattning

Återställandet av insulin frisättning vid T2D

Typ 2 diabetes (T2D) är en av våra snabbast växande sjukdomar runt om i världen, delvis till följd av olika faktorer som en stillasittande livsstil och övervikt, i kombination med genetik. Sjukdomen som i början i folkmun kallades åldersdiabetes, drabbar också medelålders och nuförtiden även yngre individer.

Sjukdomen börjar när kroppen inte kan upprätthålla blodsockernivån inom normala gränser. Detta beror på att de insulin-producerande cellerna i bukspottkörtel (β-cellerna) inte längre klarar av att tillförse kroppens olika organ med adekvat mängd insulin för att hålla blodsockernivån i balans.

I början av sjukdomen ökar kroppen insulinproduktionen i ett försök att hålla blodsockernivån nere, vilket i slutändan tröttar ut β-cellerna. Detta försök leder dock till, förr eller senare, en minskad produktion och utsöndring av insulin.

Det är känt sedan länge att förhöjda blodsockernivåer leder till dysfunktionella β-celler och med T2D som följd, men de underliggande mekanismerna är fortfarande dåligt definierade. De antidiabetiska läkemedel som finns på marknaden idag siktar in sig på att möjliggöra insulinfrisättning från β-celler samt att öka insulinkänsligheten i de perifera vävnaderna.

Ett botemedel innebär ett farmaka som kan återställa både produktionen och frisättningen av insulinet. Vägen till detta botemedel går genom en detaljerad kartläggning av de olika mekanismerna som ligger bakom produktionen och frisättningen av insulin som svar på intagna sockerarter.

I vårt arbete har vi försökt bidra till denna kartläggning genom att analysera olika G-proteinkopplade receptorer (GPCR) och hur olika substanser påverkar dessa receptorer för att signalera β-celler, och genom vilka vägar in i cellen fortplantas dessa signaler för att slutligen öka eller minska insulinfrisättningen. GPCRer är den största och mest mångsidiga gruppen av membranproteiner i våra celler med förmågan att överföra och förmedla effekten av hormoner, metaboliter, neurotransmittorer, inflammatoriska cytokiner samt läkemedel till våra celler. Vi har identifierat alla GPCRer som uttrycks i humana β-celler vilket gör det möjligt att utveckla nya läkemedel mot T2D. Parallellt har vi också tittat på hur olika substanser genom dessa receptorer påverkar cellens överlevnadsförmåga och aktivitetsnivå. Genom detta arbete har vi försökt öka förståelsen kring β-cellernas

livsduglighet och mekanismer bakomliggande dess hormonfrisättning för att slutligen kunna hitta och åtgärda defekter som uppstår i insulin produktionen och frisättningens mekanismer, vilka leder till uppkomsten av typ 2 diabetes.

I första delen av vårt arbete har vi skapat en atlas över GPCR receptorer som är gemensamma mellan människor och möss, för att kunna underlätta både vårt eget arbete, men även andra forskares arbete genom att studera de rätta GPCR-receptorerna.

I andra delen av arbetet har vi analyserat vilken roll kalk (kalcium) spelar i frisättning av insulin. Vi har samtidigt kunna visa att insulinfrisättningen sker genom snabba förändringar av cellulärt kalcium (calciumoscillationer) leder till att insulin frisätts i pulsar. Både calcium och insulin pulsalitet påverkas negativt av adenosin A(1) receptor. Våra resultat visar att A(1) hämmare har en bra effekt på insulinfrisättning from β-celler.

I delarbete tre har vi studerat hur kroppens egen substans UDP (uridin difosfat) modulerar insulinfrisättningen genom att aktivera GPCR-receptor P2Y6. Så substanser som binder och aktiverar P2Y6 har en bra effekt på insulinfrisättningen from β-celler.

I sista delen av vårt arbete har vi kartlagt hur UDP-glukos som är en naturlig ligand för GPCR-receptor P2Y14, minskar insulinfrisättningen samt hur blockaden av denna receptor förbättrar insulinfrisättningen.

Sammanfattningsvis visar resultaten i denna avhandling på att aktiveringen av P2Y6 har en bra effekt på insulinfrisättningen medan aktiveringen av vissa receptorer såsom adenosin A(1) och P2Y14 har en hämmande effekt på insulinfrisättning. Därför, aktiverare (agonister) av P2Y6 eller blockare (antagonister) av A(1) och P2Y14 kan vara attraktiva att utveckla vidare.

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This PhD has been a long journey, started during my medical studies in 2000 and finished in 2022. From beginning to end it was a wonderful journey. It was a great chance for learning new set of thinking and practising new way of experimentally generated science.

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References

Abbracchio MP, Burnstock G, Boeynaems JM, Barnard EA, Boyer JL, Kennedy C, Knight GE, Fumagalli M, Gachet C, Jacobson KA, et al. International Union of Pharmacology LVIII: update on the P2Y G protein-coupled nucleotide receptors: from molecular mechanisms and pathophysiology to therapy. Pharmacol Rev 58:281–341, 2008

Abbracchio, M. P., Boeynaems, J. M., Barnard, E. A., Boyer, J. L., Kennedy,C., Miras-Portugal, M. T., King, B. F., Gachet, C., Jacobson, K. A., Weisman,G. A., and Burnstock, G. Characterization of the UDP-glucosereceptor (re-named here the P2Y14 receptor) adds diversity to the P2Y receptor family. Trends Pharmacol. Sci 24, 52–55, 2003

Ahlqvist, E., et al., Novel subgroups of adult-onset diabetes and their association with outcomes: a data-driven cluster analysis of six variables. Lancet Diabetes Endocrinol 6(5): p. 361-369, 2018

Ahren B: Autonomic regulation of islet hormone secretion--implications for health and disease. Diabetologia 43:393-410, 2000

Amisten S, Braun OO, Bengtsson A, Erlinge D: Gene expression profiling for the identification of G-protein coupled receptors in human platelets. Thromb Res122:47-57, 2008

Amisten S, Salehi A, Rorsman P, Jones PM, Persaud SJ: An atlas and functional analysis of Gprotein coupled receptors in human islets of Langerhans. Pharmacology & therapeutics 139:359-391, 2013

Amisten S: Quantification of the mRNA expression of G protein-coupled receptors in human adipose tissue. Methods Cell Biol 132:73-105, 2016

Amisten, S., Mohammad Al-Amily, I., Soni, A., Hawkes, R., Atanes, P., Persaud, S. J., Rorsman, P., and Salehi, A. Anti-diabetic action of alltrans retinoic acid and the orphan G protein coupled receptor GPRC5C in pancreatic b-cells. Endocr. J 64, 325–338, 2017

Amisten, S., Duner, P., Asplund, O., Mohammed Al-Amily, I., Groop, L.,and Salehi, A. Activation of imidazoline receptor I2, and improved pancreatic b-cell function in human islets. J. Diabetes Complications 32, 813–818, 2018

Ashish K Rehni,Neha nautiyal, Miguel A Perez-pinzon, Kunjan R Dave Metab Brain Dis Hyperglycemia / hypoglycemia-induced mitochondrial dysfunction and cerebral ischemic damage in diabetics Ashish;30(2):437-47), 2015

Atanes, P., Ruz-Maldonado, I., Hawkes, R., Liu, B., Zhao, M., Huang, G. C., Al-Amily, I. M., Salehi, A., Amisten, S., and Persaud, S. J. Defining G protein-coupled receptor peptide ligand expressomes and signalomes in human and mouse islets. Cell. Mol. Life Sci. 75, 3039–3050, 2015

Bacher S, Kraupp O, Conca W, Raberger G. The effects of NECA (adenosine-5′Nethylcarboxamide) and of adenosine on glucagon and insulin release from the in situ isolated blood–perfused pancreas in anesthetized dogs.Naunyn Schmiedebergs Archives of Pharmacology 320 (1), 67–71, 1982

Benner C, van der Meulen T, Caceres E, Tigyi K, Donaldson CJ, Huising MO: The transcriptional landscape of mouse beta cells compared to human beta cells reveals notable species differences in long non-coding RNA and protein-coding gene expression. BMC Genomics ;15:620, 2014

Bergsten P, Hellman B. Glucose-induced amplitude regulation of pulsatile insulin secretion from individual pancreatic islets. Diabetes 42 (5), 670–674, 1993

Bertrand G, Petit P, BozemM, Henquin JC.Membrane and intracellular effects of adenosine in mouse pancreatic β-cells. American Journal of Physiology 257, E473–E478, 1989

Bertrand G, J. Chapal, R. Puech, M.M. Loubatieres-Mariani, Adenosine-5'-O-(2-thiodiphosphate) is a potent agonist at P2 purinoceptors mediating insulin secretion from perfused rat pancreas, Br J Pharmacol 102 627-630, 1991

Berts A, Gylfe E, Hellman B. Ca2+ oscillations in pancreatic islet cells secreting glucagon and somatostatin. Biochemical and Biophysical Research Communications 208 (2),644–649, 1995

Berts A, Ball A, Dryselius S, Gylfe E, Hellman B. Glucose stimulation of somatostatinproducing islet cells involves oscillatory Ca2+ signaling. Endocrinology 137 (2), 693–697, 1996a

Berts A, Ball A, Gylfe E, Hellman B. Suppression of Ca2+ oscillations in glucagonproducing α2 cells by insulin/glucose and amino acids. Biochimica et Biophysica Acta 1310 (2), 212–216, 1996b

Burnstock G. Physiology and pathophysiology of purinergic neurotransmission. Physiological Reviews 87 (2), 659–797, 2006

Buteau J, Foisy S, Joly E, Prentki M. Glucagon-like peptide 1 induces pancreatic beta-cell proliferation via transactivation of the epidermal growth factor receptor. Diabetes. 52(1):124-32, 2003

Cabrera O, Berman DM, Kenyon NS, Ricordi C, Berggren PO, Caicedo A: The unique cytoarchitecture of human pancreatic islets has implications for islet cell function. Proc Natl Acad Sci U S A 103: 2334-2339, 2006

Campbell IL, Taylor KW. Effects of adenosine, 2-deoxyadenosine and N6-phenylisopropyladenosine on rat islet function and metabolism. Biochemical Journal 204 (3), 689–696, 1982

Carter RL, Fricks IP, Barrett MO, Burianek LE, Zhou Y, Ko H, Das A, Jacobson KA, Lazarowski ER, Harden TK. Quantification of Gi-mediated inhibition of adenylyl cyclase activity reveals that UDP is a potent agonist of the human P2Y14 receptor. Mol Pharmacol 76:1341–1348, 2009

Chambers JK, Macdonald LE, Sarau HM, Ames RS, Freeman K, Foley JJ, Zhu Y, McLaughlin MM, Murdock P, McMillan L, et al. A G protein-coupled receptor for UDP-glucose, JBC 275(15):10767-71, 2000

Chapal J, Loubatières-Mariani M, Petit P, Roye M. Evidence for an A2-subtype adenosine receptor on pancreatic glucagon secreting cells. British Journal of Pharmacology 86(3), 565–569, 1985

Chen Y.J., K.W. Hsu, Y.L. Chen, Acute glucose overload potentiates nitric oxide production in lipopolysaccharide-stimulated macrophages: the role of purinergic receptor activation, Cell Biol Int 30; 817-822, 2006

Chow RH, Lund PE, Löser S, Panten U, Gylfe E. Coincidence of early glucose-induced depolarization with lowering of cytoplasmic Ca2+ in mouse pancreatic β-cells. Journal of Physiology 485 (Pt 3), 607–617, 1995

Communi D, M. Parmentier, J.M. Boeynaems, Cloning, functional expression and tissue distribution of the human P2Y6 receptor, Biochem Biophys Res Commun 222; 303-308, 1996

Costes, S. V., Daelemans, D., Cho, E. H., Dobbin, Z., Pavlakis, G., and Lockett,S. Automatic and quantitative measurement of protein-protein colocalization in live cells. Biophys. J. 86, 3993–4003, 2004

Dai C, Brissova M, Hang Y, Thompson C, Poffenberger G, Shostak A, Chen Z, Stein R, Powers AC: Islet-enriched gene expression and glucose-induced insulin secretion in human and mouse islets. Diabetologia;55:707-718, 2012

Das, A., Ko, H., Burianek, L. E., Barrett, M. O., Harden, T. K., and Jacobson, K. A. Human P2Y(14) receptor agonists: truncation of the hexose moiety of uridine-5'-diphosphoglucose and its replacement with alkyl and aryl groups. J. Med. Chem. 53, 471–480, 2010

DeFronzo, R.A., et al., Type 2 diabetes mellitus. Nat Rev Dis Primers, 1: p. 15019, 2015

De Marinis YZ, Salehi A, Ward CE, Zhang Q, Abdulkader F, Bengtsson M, Braha O, Braun M, Ramracheya R, Amisten S, Habib AM, Moritoh Y, Zhang E, Reimann F, Rosengren AH, Shibasaki T, Gribble F, Renstrom E, Seino S, Eliasson L, Rorsman P: GLP-1 inhibits and adrenaline stimulates glucagon release by differential modulation of N- and L-type Ca2+ channel-dependent exocytosis. Cell Metab 11:543-553, 2010

Dunér, P., Al-Amily, I. M., Soni, A., Asplund, O., Safi, F., Storm, P., Groop, L., Amisten, S., and Salehi, A. AdhesionG protein-coupled receptor G1 (ADGRG1/GPR56) and pancreatic b-cell function. J. Clin. Endocrinol. Metab. 101, 4637–4645, 2016

Ellard SC et al, Diabetologia 2008 Best practice guidelines for the molecular genetic diagnosis of maturity-onset diabetes of the young, 51(4):546-53.

Fernandez-Alvarez J, D. Hillaire-Buys, M.M. Loubatieres-Mariani, R. Gomis, P. Petit, P2 receptor agonists stimulate insulin release from human pancreatic islets, Pancreas 22 69-71, 2001

Fischer B, A. Chulkin, J.L. Boyer, K.T. Harden, F.P. Gendron, A.R. Beaudoin, J. Chapal, D. Hillaire-Buys, P. Petit, 2-thioether 5'-O-(1-thiotriphosphate) adenosine derivatives as new insulin secretagogues acting through P2Y-Receptors, J Med Chem 42: 3636-3646, 1999

Flower DR. Modelling G-protein-coupled receptors for drug design. Biochimica et biophysica acta. ;1422(3):207-34, 1999

Fredholm BB, Ijserman AP, Jacobson KA, Klotz KN, Linden J. International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. Pharmacological Reviews 53 (84), 527–552, 2001

Fricks K O H I, Ivanov AA, Harden TK, Jacobson KA. Structure-activity relationship of uridine 5′-diphosphoglucose analogues as agonists of the human P2Y14 receptor. J Med Chem 50:2030–2039, 2007

Get GO GM: The maintenance of human normal cells and tumor cells in continuous culture: I. Preliminary report: cultivation of mesoblastic tumors and normal tissue and notes on methods of cultivation. Am J Cancer 27:45–76, 1936

Grapengiesser E, H. Dansk, B. Hellman, Pulses of external ATP aid to the synchronization of pancreatic beta-cells by generating premature Ca(2+) oscillations, Biochem Pharmacol 68 667-674, 2004

Grapengiesser E, Gylfe E, Hellman B. Cyclic AMP as a determinant for glucose induction of fast Ca2+ oscillations in isolated pancreatic β-cells. Journal of Biological Chemistry 226 (19), 12207–12210, 1991

Grapengiesser E, Dansk H, Hellman B. Synchronization of pancreatic β-cell rhythmicity after glucagon induction of Ca2+ transients. Cell Calcium 34 (1), 49–53, 2003

Grapengiesser E, Salehi A, Qader SS, Hellman B. Glucose induces glucagon release pulses antisynchronouswith insulin and sensitive to purinoceptor inhibition. Endocrinology 147 (7), 3472–3477, 2006

Gross R, Bertrand G, Ribes G, Petit P, Loubatieres-Mariani M. Epinephrine potentiates adenosine-stimulating effect on glucagon secretion. American Journal of Physiology 252 (3, Pt 1), E426–E430, 1987

Grynkiewicz G, Poenie M, Tsien RY. A new generation of Ca2+ indicators with greatly improved fluorescence properties. Journal of Biological Chemistry 260 (6), 3440–3450, 1985

Gylfe E, Grapengiesser E, Hellman B. Propagation of cytoplasmic Ca2+ oscillations in clusters of pancreatic β-cells exposed to glucose. Cell Calcium 12, 229–240, 1991

Hahn HJ, Hellman B, Lernmark Å, Sehlin J, Täljedal IB. The pancreatic β-cell recognition of insulin secretagogues. Influence of neuraminidase treatment on the release of insulin and the islet content of insulin, sialic acid and cyclic adenosine 3′:5′-monophosphate. Journal of Biological Chemistry 249 (16), 5275–5284, 1974

Hazama A., S. Hayashi, Y. Okada, Cell surface measurements of ATP release from single pancreatic beta cells using a novel biosensor technique, Pflugers Arch 437 31-35, 1998

Heding LH. Determination of free and antibody-bound insulin in insulin treated diabetic patients. Hormone and Metabolic Research 1 (3), 145–146, 1969.

Hellman B. Studies in obese-hyperglycemic mice. Annals of the New York Academy of Sciences 131 (1), 541–558, 1965

Hellman B. Calcium transport in pancreatic β-cells: Implication for glucose regulation of insulin release. Diabetes/Metabolism Reviews 2 (3–4), 215–241, 1986

Hellman B, Gylfe E, Bergsten P, Grapengiesser E, Lund P-E, Berts A, Tengholm A, Pipeleers D, Ling Z. Glucose induces oscillatory Ca2+ signalling and insulin release in human pancreatic beta cells. Diabetologia 37 (Suppl 2), S11–S20, 1994

Hellman B, Dansk H, Grapengiesser E. Pancreatic β-cells communicate via intermittent release of ATP. American Journal of Physiology-Endocrinology and Metabolism 286 (5), E759–E765, 2004

Hillaire-Buys D, Bertrand G, Gross R, Loubatières-Mariani MM. Evidence for an inhibitory A1 subtype adenosine receptor on pancreatic insulin-secreting cells. European Journal of Pharmacology 136 (1), 109–112, 1987

Hillaire-Buys D, Chapal J, Bertrand G, Petit P, Loubatières-Mariani MM. Purinergic recptors on insulin-secreting cells. Fundamental & Clinical Pharmacology 8 (2), 117–127, 1994

Hohmeier, H. E., and Newgard, C. B. Cell lines derived from pancreatic Endocrinol. 228, 121–128 islets. Mol. Cell, 2004

Huang GC, Zhao M, Jones P, Persaud S, Ramracheya R, Lobner K, Christie MR, Banga JP, Peakman M, Sirinivsan P, Rela M, Heaton N, Amiel S: The development of new density gradient media for purifying human islets and islet-quality assessments. Transplantation 77:143-145, 2004

Hutton J.C, E.J. Penn, M. Peshavaria, Low-molecular-weight constituents of isolated insulin-secretory granules. Bivalent cations, adenine nucleotides and inorganic phosphate, Biochem J 210 297-305, 1983

Ismail NA, El Denshary EE, Montague W. Adenosine and the regulation of insulin secretion by isolated rats islets of Langerhans. Biochemical Journal 164 (2), 409–413, 1977

JE (Eds.),Methods in Neurosciences, vol. 20. Academic Press, USA, pp. 336–376, 1994

Jimenez-Feltstrom, J., Lundquist, I., and Salehi, A. Glucose stimulates the expression and activities of nitric oxide synthases in incubated rat islets: An effect counteracted by GLP-1 through the cyclic AMP/PKA pathway. Cell Tissue Res. 319, 221–230, 2005

Jing X, Li DQ, Olofsson CS, Salehi A, Surve VV, Caballero J, Ivarsson R, Lundquist I, Pereverzev A, Schneider T, Rorsman P, Renström E. CaV2.3 calcium channels control second-phase insulin release. Journal of Clinical Investigation 115 (1), 146–154, 2005

Johansson SM, Salehi A, Sandström ME, Westerblad H, Lundquist I, Carlsson PO, Fredholm BB, Katz A. A1 receptor deficiency causes increased insulin and glucagon secretion in mice. Biochemical Pharmacology 74 (11), 1628–1635, 2007

Jones PM, Salmon DM, Howell SL: Protein phosphorylation in electrically permeabilized islets of Langerhans. Effects of Ca2+, cyclic AMP, a phorbol ester and noradrenaline. Biochem J 254:397-403, 1988

Jones, A. L. ; Goetsch, A. L. ; Stokes, S. R. ; Colberg, M., Intake and digestion in cattle fed warm- or cool-season grass hay with or without supplemental grain J. Anim. Sci., 66 (1): 194-203, 1988

Jones PM PS: Textbook of Diabetes. In Textbook of Diabetes Holt G, Flyvberg, Ed., Blackwell Scientific Press, UK, p. pp87-103, 2010

Kim A, Miller K, Jo J, Kilimnik G, Wojcik P, Hara M: Islet architecture: A comparative study. Islets 1:129-136, 2009

Kreda, S.M, S.F. Okada, C.A. van Heusden, W. O'Neal, S. Gabriel, L. Abdullah, C.W. Davis, R.C. Boucher, E.R. Lazarowski, Coordinated release of nucleotides and mucin from human airway epithelial Calu-3 cells, J Physiol 584 ;245-259, 2007

Kreda S.M, L. Seminario-Vidal, C.V. Heusden, E.R. Lazarowski, Thrombin-promoted release of UDP-glucose from human astrocytoma cells, Br J Pharmacol 153(7):1528-37, 2008

Krischer JP et al, Predicting Islet Cell Autoimmunity and Type 1 Diabetes: An 8-Year TEDDY Study Progress Report,Diabetes Care. Jun;42(6):1051-1060, 2019

Kumar, R., Balhuizen, A., Amisten, S., Lundquist, I., and Salehi, A. Insulinotropic and antidiabetic effects of 17b-estradiol and the GPR30 agonist G-1 on human pancreatic islets. Endocrinology 152, 2568–2579, 2011

Kutlu B, Burdick D, Baxter D, Rasschaert J, Flamez D, Eizirik DL, Welsh N, Goodman N, Hood L: Detailed transcriptome atlas of the pancreatic beta cell. BMC Med Genomics ;2:317, 2009

Lacey RJ, Berrow NS, London NJ, Lake SP, James RF, Scarpello JH, Morgan NG: Differential effects of beta-adrenergic agonists on insulin secretion from pancreatic islets isolated from rat and man. J Mol Endocrinol;5:49-54, 1990

Lang J, Molecular mechanisms and regulation of insulin exocytosis as a paradigm of endocrine secretion, European journal of biochemistry / FEBS 259 3-17, 1999

Lazarowski ER, Harden TK.. UDP-Sugars as Extracellular Signaling Molecules: Cellular and Physiologic Consequences of P2Y14 Receptor Activation. Mol Pharmacol. 88(1):151-60, 2015

Lazarowski E.R, T.K. Harden, Quantitation of extracellular UTP using a sensitive enzymatic assay, Br J Pharmacol 127;1272-1278, 1999

Lee, B. C., Cheng, T., Adams, G. B., Attar, E. C., Miura, N., Lee, S. B., Saito, Y., Olszak, I.,Dombkowski, D., Olson, D. P., Hancock, J., Choi, P. S., Haber, D. A., Luster, A. D., and Scadden, D. TP2Y-like receptor, GPR105 (P2Y14), identifies and mediates chemotaxis of bone-marrow hematopoietic stemcells. Genes Dev. 17, 1592–1604, 2003

Léon C, M. Freund, O. Latchoumanin, A. Farret, P. Petit, J.-P. Cazenave, C. Gachet, The P2Y1 receptor is involved in the maintenance of glucose homeostasis and in insulin secretion in mice, Purinergic Signalling 1;145-151, 2005

Liang Y & Matschinsky FM, Annu Rev Nutr. Mechanisms of action of nonglucose insulin secretagogues;14:59-81, 1994

Li GD, Milani D, Dunne MJ, Pralong WF, Theler JM, Petersen OH, Wollheim CB: Extracellular ATP causes Ca2(+)-dependent and -independent insulin secretion in RINm5F cells. Phospholipase C mediates Ca2+ mobilization but not Ca2+ influx and membrane depolarization. J Biol Chem;266:3449-3457, 1991

Lindstrom E, M. Bjorkquist, A. Boketoft, D. Chen, C.M. Zhao, K. Kimura, R. Hakanson, Release of histamine and pancreastatin from isolated rat stomach ECL cells, Inflamm Res 46 Suppl 1 S109-110, 1997

Liu B, Hassan Z, Amisten S, King AJ, Bowe JE, Huang GC, Jones PM, Persaud SJ: The novel chemokine receptor, G-protein-coupled receptor 75, is expressed by islets and is coupled to stimulation of insulin secretion and improved glucose homeostasis. Diabetologia 56:2467-2476, 2013

Li X, Zhong K, Guo Z, Zhong D, Chen X: Fasiglifam (TAK-875) Inhibits Hepatobiliary Transporters: A Possible Factor Contributing to Fasiglifam-Induced Liver Injury. Drug Metab Dispos;43:1751-1759, 2015

Loubatieres-Mariani M.M, J. Chapal, F. Lignon, G. Valette, Structural specificity of nucleotides for insulin secretory action from the isolated perfused rat pancreas, Eur J Pharmacol 59, 277-286, 1979

Malmsjo M, M. Adner, T.K. Harden, W. Pendergast, L. Edvinsson, D. Erlinge, The stable pyrimidines UDPbetaS and UTPgammaS discriminate between the P2 receptors that mediate vascular contraction and relaxation of the rat mesenteric artery, Br J Pharmacol 131. 51-56, 2005

Mamedova L.K, B.V. Joshi, Z.G. Gao, I. von Kugelgen, K.A. Jacobson, Diisothiocyanate derivatives as potent, insurmountable antagonists of P2Y6 nucleotide receptors, Biochem Pharmacol 67.1763-1770, 2004

Marioni JC, Mason CE, Mane SM, Stephens M, Gilad Y: RNA-seq: an assessment of technical reproducibility and comparison with gene expression arrays. Genome Res 18:1509-1517, 2008

Meister J, Le Duc D, Ricken A, Burkhardt R, Thiery J, Pfannkuche H, Polte T, Grosse J, Schöneberg T, Schulz A. The G protein-coupled receptor P2Y14 influences insulin release and smooth muscle function in mice. J Biol Chem 289:23353–23366. receptor for UDP-glucose. J Biol Chem 275:10767–10771, 2014

Meister, J., Le Duc, D., Ricken, A., Burkhardt, R., Thiery, J., Pfannkuche, H., Polte, T., Grosse, J., Schöneberg, T., and Schulz, A. The G protein- coupled receptor P2Y14 influences insulin release and smooth muscle function in mice. J. Biol. Chem. 289, 23353–23366, 2014

Mohammed Al-Amily, I. M., Duner, P., Groop, L., and Salehi, A. The functional impact of G protein-coupled receptor 142 (Gpr142) on pancreatic b-cell in rodent. Pflugers Arch. 471, 633–645,2015

Moon, J.H., S.H. Kwak, and H.C. Jang, Prevention of type 2 diabetes mellitus in women with previous gestational diabetes mellitus. Korean J Intern Med, 32(1): p. 26-41, 2017

Moore DJ, Murdock PR, Watson JM, Faull RL, Waldvogel HJ, Szekeres PG, Wilson S, Freeman KB, Emson PC. GPR105, a novel Gi/o-coupled UDP-glucose receptor expressed on brain glia and peripheral immune cells, is regulated by immunologic challenge: possible role in neuroimmune function. Brain Res Mol Brain Res 118:10–23, 2003

Muhammed SJ , Lundquist,A. Salehi, Diabeted, Obesity and Metabolism Pancreatic β-cell dysfunction, expression of iNOS and the effect of phosphodiesterase inhibitors in human pancreatic islets of type 2 diabetes 2012

Nagalakshmi U, Wang Z, Waern K, Shou C, Raha D, Gerstein M, Snyder M: The transcriptional landscape of the yeast genome defined by RNA sequencing. Science 320:1344-1349, 2008

Nilsson J, L.M. Nilsson, Y.W. Chen, J.D. Molkentin, D. Erlinge, M.F. Gomez, High glucose activates nuclear factor of activated T cells in native vascular smooth muscle, Arterioscler Thromb Vasc Biol 26.794-800, 2006

Novak, I. Purinergic receptors in the endocrine and exocrine pancreas. Purinergic Signal. 4, 237–253, 2008

Nunemaker CS, Wasserman DH, McGuiness OP, Sweet IR, Teague JC, Satin LS. Insulin secretion in the conscious mouse is biphasic and pulsatile. American Journal of Physiology-Endocrinology and Metabolism 290 (3), E523–E529, 2006.

Oh da Y, Olefsky JM: G protein-coupled receptors as targets for anti-diabetic therapeutics. Nat Rev Drug Discov 2016;15:161-1728. Regard JB, Sato IT, Coughlin SR: Anatomical profiling of G protein-coupled receptor expression. Cell 135:561-57116, 2008

Olah & Stiles, the role of receptor structure in determining adenosine receptor activity, pharmacol, Ther, 85(2), 55-57, 2000

Opara EC, Atwater I, Go VL. Characterization and control of pulsatile secretion of insulin and glucagon. Pancreas 3 (4), 484–487, 1988

Parodi J, C. Flores, C. Aguayo, M.I. Rudolph, P. Casanello, L. Sobrevia, Inhibition of nitrobenzylthioinosine-sensitive adenosine transport by elevated D-glucose involves activation of P2Y2 purinoceptors in human umbilical vein endothelial cells, Circ Res 90 , 570-577, 2002

Pasquali L, Gaulton KJ, Rodriguez-Segui SA, Mularoni L, Miguel-Escalada I, Akerman I, Tena JJ, Moran I, Gomez-Marin C, van de Bunt M, Ponsa-Cobas J, Castro N, Nammo T, Cebola I, Garcia- Hurtado J, Maestro Pattou M.A, F, Piemonti L, Berney T, Gloyn AL, Ravassard P, Gomez-Skarmeta JL, Muller F, McCarthy MI, Ferrer J: Pancreatic islet enhancer clusters enriched in type 2 diabetes riskassociated variants. Nat Genet 46:136-143, 2014

Persaud SJ: Function and expression of melatonin receptors on human pancreatic islets. J Pineal Res24. Rask-Andersen M, Almen MS, Schioth HB: Trends in the exploitation of novel drug targets. Nat Rev Drug Discov 10:579-590, 2011

Petit P, G. Bertrand, W. Schmeer, J.C. Henquin, Effects of extracellular adenine nucleotides on the electrical, ionic and secretory events in mouse pancreatic beta-cells, Br J Pharmacol 98 ,875-882, 1989

Pfaffl, M. W. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 29, e45, 2001

Poulsen C.R, K. Bokvist, H.L. Olsen, M. Hoy, K. Capito, P. Gilon, J. Gromada, Multiple sites of purinergic control of insulin secretion in mouse pancreatic beta-cells, Diabetes 48,2171-2181, 1999

Pørksen N. The in vivo regulation of pulsatile insulin secretion. Diabetologia 45 (1), 3–20, 2002

Qader S.S, R. Hakanson, J.F. Rehfeld, I. Lundquist, A. Salehi, Proghrelin-derived peptides influence the secretion of insulin, glucagon, pancreatic polypeptide and somatostatin: a study on isolated islets from mouse and rat pancreas, Regul Pept 146, 230-237, 2008

Qader S.S, J. Jimenez-Feltstrom, M. Ekelund, I. Lundquist, A. Salehi, Expression of islet inducible nitric oxide synthase and inhibition of glucose-stimulated insulin release after long-term lipid infusion in the rat is counteracted by PACAP27, Am J Physiol Endocrinol Metab 292, E1447-1455, 2007

Ralevic V, G. Burnstock, Receptors for purines and pyrimidines, Pharmacological Reviews 50, 413-492, 1998

Ramracheya RD, Muller DS, Squires PE, Brereton H, Sugden D, Huang GC, Amiel SA, Jones PM, shanta J persuad. Function and expression of melatonin receptors on human pancreatic islets, J pineal res 44(3):273-9, 2008

Regard JB, Kataoka H, Cano DA, Camerer E, Yin L, Zheng YW, Scanlan TS, Hebrok M, Coughlin SR: Probing cell type-specific functions of Gi in vivo identifies GPCR regulators of insulin secretion. J Clin Invest 117:4034-4043 , 2007

Reshkin, S. J., Bellizzi, A., Caldeira, S., Albarani, V., Malanchi, I., Poignee, M., Alunni-Fabbroni, M., Casavola, V., and Tommasino, M. Na+/H+ exchanger-dependent intracellular alkalinization is an early event in malignant transformation and plays an essential role in the development of subsequent transformation-associated phenotypes. FASEB J. 14, 2185–2197, 2000

Revathy Carnagarin et al, Autonomic Regulation of Glucose Homeostasis: a Specific Role for Sympathetic Nervous System Activation, Curr Diab Rep 19;18(11):107, 2018

Ritzel RA, Veldhuis JD, Butler PC. Glucose stimulates pulsatile insulin secretion fromhuman pancreatic islets by increasing secretory burst mass: Dose-response relationships. Journal of Clinical Endocrinology and Metabolism 88 (2), 742–747, 2003.

Robas N, O'Reilly M, Katugampola S, Fidock M. Maximizing serendipity: strategies for identifying ligands for orphan G-protein-coupled receptors. Current opinion in pharmacology. 3(2):121-6), 2003

Robaye B, J.M. Boeynaems, D. Communi, Slow desensitization of the human P2Y6 receptor, Eur J Pharmacol 329, 231-236, 1999

Salehi A, S.S. Qader, E. Grapengiesser, B. Hellman, Inhibition of purinoceptors amplifies glucose-stimulated insulin release with removal of its pulsatility, Diabetes 54, 2126-2131, 2005

Salehi A, Carlberg M, Henningsson R, Lundquist I. Islet constitutive nitric oxide synthase: biochemical determination and regulatory function. Am J Physiol Cell Physiol 270:C1634-41, 1996

Salehi A, Qader SS, Grapengiesser E, Hellman B. Inhibition of purinoceptors amplifies glucose-stimulated insulin release with removal of its pulsatility. Diabetes 54 (7), 2126–2131, 2005

Salehi A, Qader SS, Grapengiesser E, Hellman B. Pulses of somatostatin are slightly delayed compared with insulin and antisynchronous to glucagon. Regulatory Peptides 144 (1–3), 43–49, 2007

Salehi A, Qader SS, Grapengiesser E, Hellman B. Inhibition of purinoceptors amplifies glucose-stimulated insulin release with removal of its pulsatility. Diabetes 54 (7),2126–2131, 2005

Salehi A, Qader SS, Grapengiesser E, Hellman B. Pulses of somatostatin are slightly delayed compared with insulin and antisynchronous to glucagon. Regulatory Peptides 144 (1–3),43–49, 2007

Seino S & Shibasaki T, Physiol Rev 85: 1303-1342, 2005

Schuit FC & Pipeleers DG. Regulation of adenosine 3′, 5′-monophosphate levels in the pancreatic B-cell. Endocrinology 117 (3), 834–840, 1985

Seino S & Shibasaki T, PKA-dependent and PKA-independent pathways for cAMP-regulated exocytosis Physiol Rev 85, 2005

Silvestre RA, Rodriguez-Gallardo J, Egido EM, Marco J. Interrelationship among insulin,glucagon and somatostatin secretory responses to exendin-4 in the perfused pancreas. European Journal of Pharmacology 469 (1–3), 195–200, 2003

Sharp, G. W. Mechanisms of inhibition of insulin release. Am. J.Physiol. 271, C1781–C1799, 1996

Silvestre RA, Rodriguez-Gallardo J, Egido EM, Marco J. Interrelationship among insulin, glucagon and somatostatin secretory responses to exendin-4 in the perfused pancreas. European Journal of Pharmacology 469 (1–3), 195–200, 2003

Solini A, C. Iacobini, C. Ricci, P. Chiozzi, L. Amadio, F. Pricci, U. Di Mario, F. Di Virgilio, G. Pugliese, Purinergic modulation of mesangial extracellular matrix production: role in diabetic and other glomerular diseases, Kidney Int 67, 875-885, 2005

Soni A, Amisten S, Rorsman P, Salehi A: GPRC5B a putative glutamate-receptor candidate is negative modulator of insulin secretion. Biochem Biophys Res Commun 441(3):643-648, 2013

Tatur S, N. Groulx, S.N. Orlov, R. Grygorczyk, Ca2+-dependent ATP release from A549 cells involves synergistic autocrine stimulation by coreleased uridine nucleotides, J Physiol 584, 419-435, 2007

Schuit FC, Pipeleers DG. Regulation of adenosine 3′, 5′-monophosphate levels in the pancreatic B-cell. Endocrinology 117 (3), 834–840, 1985

Squires PE, James RF, London NJ, Dunne MJ: ATP-induced intracellular Ca2+ signals in isolated human insulin-secreting cells. Pflugers Arch 427:181-183, 1994

Srivastava A, Yano J, Hirozane Y, Kefala G, Gruswitz F, Snell G, Lane W, Ivetac A, Aertgeerts K, Nguyen J, Jennings A, Okada K: High-resolution structure of the human GPR40 receptor bound to allosteric agonist TAK-875. Nature 513:124-127, 2014

Tuch BE: Clinical use of GLP-1 agonists and DPP4 inhibitors. Pancreatology 2016;16:8-911, 2016

Tuduri E, Filiputti E, Carneiro EM, Quesada I. Inhibition of Ca2+ signaling and glucagon secretion in mouse pancreatic α-cells by extracellular ATP and purinergic receptors. American Journal of Physiology-Endocrinology and Metabolism 294 (5), E952–E960, 2008

Unger RH, Orci L. Possible roles of the pancreatic D-cell in the normal and diabetic states. Diabetes 26 (3), 241–244, 1977

Veldhuis JD, Johnson ML. Cluster analysis: A simple, versatile and robust algorithm for endocrine pulse detection. American Journal of Physiology-Endocrinology and Metabolism 250 (4 Pt 1), E486–E493, 1986

Veldhuis JD, JohnsonML, Faunt LM, Seneta E. Assessing temporal coupling between twoor among three or more neuroendocrine pulse trains (chapter 17). In: Conn, PM, Levine,

Welsh M, Andersson A. Adenosine uptake by isolated mouse pancreatic islets. Biochemical Pharmacology 30 (15), 2075–2080, 1981

Xu J, Morinaga H, Oh D, Li P, Chen A, Talukdar S, Mamane Y, Mancini JA, Nawrocki AR, Lazarowski E, et al. GPR105 ablation prevents inflammation and improves insulin sensitivity in mice with diet-induced obesity. J Immunol 189:1992–1999, 2012

Zhao S, Fung-Leung WP, Bittner A, Ngo K, Liu X: Comparison of RNA-Seq and microarray in transcriptome profiling of activated T cells. PLoS On 9:e78644, 2014

Zhang, E., Mohammed Al-Amily, I., Mohammed, S., Luan, C., Asplund, O., Ahmed, M., Ye, Y., Ben-Hail, D., Soni, A., Vishnu, N., Bompada, P., De Marinis, Y., Groop, L., Shoshan-Barmatz, V., Renstrom, E., et al. Preserving insulin secretion in diabetes by inhibiting VDAC1 overexpression and surface translocation in b cells. Cell Metab. 29, 64–77e66, 2019

Zhao F., M., Huang, G. C., Salehi, A., and Persaud, S. J. A comparative analysis of human and mouse islet G-protein coupled receptor expression.Sci. Rep. 7, 46600, 2017

Zimmermann H.Extracellular metabolism of ATP and other nucleotides. Naunyn-Schmiedebergs Archives of Pharmacology 362 (4–5), 299–309, 2000

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