Regulation of insulin secretion - Role of pancreatic NMDA receptors in beta cell function

Inaugural-Dissertation

zur Erlangung des Doktorgrades
der Mathematisch-Naturwissenschaftlichen Fakultät
der Heinrich-Heine-Universität Düsseldorf

vorgelegt von

Silke Otter
aus Duisburg

Düsseldorf, Februar 2016

aus dem Institut für Stoffwechselphysiologie
der Heinrich-Heine-Universität Düsseldorf

Gedruckt mit der Genehmigung der
Mathematisch-Naturwissenschaftlichen Fakultät der
Heinrich-Heine-Universität Düsseldorf

Referent: Prof. Dr. Eckhard Lammert

Korreferent: Prof. Dr. Philipp A. Lang

Tag der mündlichen Prüfung: 05. Juli 2016

Table of contents

Zusammenfassung

Pankreatische Inseln stellen den endokrinen Teil des Pankreas dar und bestehen aus bis zu fünf unterschiedlichen endokrinen Zelltypen. Die Blutglukosekonzentration im Körper ändert sich nur innerhalb eines sehr geringen Bereichs und wird streng kontrolliert primär durch die Sekretion und Wirkung von pankreatischen Hormonen (Glukosehomöostase). Unter den pankreatischen endokrinen Zelltypen haben Betazellen eine besondere Funktion, da sie Insulin, ein Blutzucker senkendes Hormon produzieren und sekretieren. Inadäquate Insulinsekretion, ausgelöst durch eine Fehlfunktion der Betazellen, kann zu schwerwiegenden akuten sowie Langzeit Konsequenzen führen. Eine Insulinsekretion, die zu gering ist um den körperlichen Bedarf an Insulin zu decken, resultiert in erhöhten Blutglukosewerten (Hyperglykämie) was letztendlich zu der Stoffwechselkrankheit Diabetes mellitus führen kann. Ist die Menge an sekretiertem Insulin hingegen unverhältnismäßig hoch, kann die Blutglukosekonzentration unter den physiologischen Bereich fallen (Hypoglykämie).

Um eine adäquate Menge an Insulin zu sekretieren, müssen Betazellen Nährstoffe wahrnehmen sowie eine Vielzahl von Signalmolekülen, wie zum Beispiel Neurotransmitter und andere pankreatische Hormone, empfangen. Während meiner Doktorarbeit habe ich an der Regulation der Insulinsekretion von pankreatischen Betazellen gearbeitet. In der Vergangenheit konnten Studien zeigen, dass der exzitatorische Neurotransmitter Glutamat eine wichtige Rolle für die Funktion und das Überleben von pankreatischen Inseln spielt. Glutamat kann die Hormonsekretion des Pankreas, sowohl intrazellulär als auch extrazellulär, beispielsweise durch Bindung an Glutamatrezeptoren beeinflussen. NMDA Rezeptoren (NMDAR) sind ionotrope Glutamatrezeptoren, welche weitreichend im zentralen Nervensystem exprimiert sind. Es ist bekannt, dass NMDAR auch in pankreatischen Inseln exprimiert sind, allerdings wurde deren Rolle bezüglich der pankreatischen Hormonsekretion, insbesondere Insulinsekretion, noch nicht genau erforscht. Deshalb lag das Hauptaugenmerk meiner Doktorarbeit auf der Untersuchung der Rolle pankreatischer NMDAR in der Funktion von Betazellen. Unter Zuhilfenahme von pharmakologischen sowie auch genetischen Methoden konnte unsere Arbeitsgruppe zeigen, dass pankreatische NMDAR eine Rolle in der Regulation der Insulinsekretion und Glukosekontrolle spielen.

Insbesondere konnten wir herausfinden, dass die Inhibierung von NMDAR in isolierten menschlichen wie auch Mausinseln die Glukose stimulierte Insulinsekretion erhöhte. Die Verabreichung des NMDAR Antagonisten Dextromethorphan (DXM) an Wildtypmäuse erhöhte deren Glukosetoleranz. Die Langzeitbehandlung eines Mausmodels für Typ 2 Diabetes mit DXM verbesserte sowohl die Glukosetoleranz der Mäuse, als auch den Insulingehalt der Inseln und die Inselzellmasse.
Des Weiteren konnten wir durch eine Vielzahl von genetischen und pharmakologischen Experimenten, als auch durch eine Kombination beider, einen Teil des zu Grunde liegenden molekularen Mechanismus aufdecken, wie NMDAR daran beteiligt sind, die Insulinsekretion zu regulieren. Zudem konnte in zwei „Proof-of-concept“ Studien gezeigt werden, dass sowohl eine Einzeldosis DXM, als auch die Gabe von DXM zusammen mit einem DPP4 Inhibitor, die Glukosetoleranz sowie die Insulinkonzentrationen im Serum während eines Glukosetoleranztest in Typ 2 Diabetikern verbessert.

Unsere Ergebnisse weisen darauf hin, dass pankreatische NMDAR in die Regulation der Insulinsekretion und Blutglukosekontrolle involviert sind und zeigen zudem die Möglichkeit auf, dass pankreatische NMDAR als „Drugtargets“ in der unterstützenden Behandlung des Diabetes mellitus dienen können.

Abstract

Pancreatic islets represent the endocrine part of the pancreas and consist of up to five different endocrine cell types. In the body the blood glucose concentration is always kept within a narrow range and is tightly controlled primarily by the secretion and action of pancreatic hormones (glucose homeostasis). Among the pancreatic endocrine cell types beta cells are of particular importance as they produce and release the blood glucose lowering hormone insulin. Inappropriate insulin secretion resulting from the dysfunction of pancreatic beta cells can lead to serious acute and long-term consequences. Insulin secretion that is too low to meet the body's needs results in elevated blood glucose concentrations (hyperglycemia) which eventually can give rise to the metabolic disorder diabetes mellitus. On the other hand, when the amount of secreted insulin is inappropriately high, blood glucose concentrations can fall below the physiological range (hypoglycemia).

In order to appropriately secrete insulin, beta cells need to sense nutrients and receive a variety of signaling molecules including neurotransmitters and other pancreatic hormones. During my PhD thesis I worked on the regulation of insulin secretion from pancreatic beta cells. In the past, studies have revealed that the excitatory neurotransmitter glutamate plays an important role in pancreatic islet function and survival. Glutamate can influence pancreatic hormone secretion both intracellularly as well as extracellularly for example via binding to glutamate receptors. NMDA receptors (NMDARs) are ionotropic glutamate receptors that are broadly expressed in the central nervous system. It is known that NMDARs are also expressed in pancreatic islets, but their role in pancreatic hormone secretion, and in particular insulin secretion, remained elusive. Therefore, the primary aim of my PhD thesis was the investigation of the role of pancreatic NMDARs in beta cell function. Using pharmacological as well as genetic approaches, our research group could show that pancreatic NMDARs are involved in the regulation of insulin secretion and glucose control.

In particular we found that inhibition of NMDARs in isolated human and mouse pancreatic islets increased glucose-stimulated insulin secretion. Application of dextromethorphan (DXM), an NMDAR antagonist, enhanced the glucose tolerance of wildtype mice. Long-term treatment of a type 2 diabetic mouse model with DXM improved glucose tolerance as well as islet insulin content and islet cell mass.
Furthermore, using a variety of genetic and pharmacological experiments or a combination of both we could shed light on the underlying molecular mechanism of how NMDARs are involved in the regulation of insulin secretion. In addition two proof-of-concept studies revealed that both, single dose administration of DXM as well as DXM in combination with a DPP4 inhibitor have an improving effect on glucose control as well as serum insulin concentrations during a glucose tolerance test in type 2 diabetics.

Our results indicate that pancreatic NMDARs are involved in the regulation of insulin secretion and blood glucose control and reveal the possibility that pancreatic NMDARs may serve as drug targets for adjunct treatment of diabetes mellitus.

1 Annotations to this thesis

This thesis consists of the following four independent publications (chronological order):

- J. Marquard, A. Welters, T. Buschmann, W. Barthlen, S. Vogelgesang, D. Klee, M. Krausch, A. Raffel, **S. Otter**, L. Piemonti, E. Mayatepek, T. Otonkoski, E. Lammert, T. Meissner - Association of exercise-induced hyperinsulinaemic hypoglycaemia with *MCT1*-expressing insulinoma - Diabetologia 2013 (Marquard et al., 2013)
  Original research article

- J. Marquard*, **S. Otter***, A. Welters*, A. Stirban, A. Fischer, J. Eglinger, D. Herebian, O. Kletke, M. Skelin Klemen, A. Stozer, S. Wnendt, L. Piemonti, M. Köhler, J. Ferrer, B. Thorens, F. Schliess, M.S. Rupnik, T. Heise, P.O. Berggren, N. Klöcker, T. Meissner, E. Mayatepek, D. Eberhard, M. Kragl, E. Lammert - Characterization of pancreatic NMDA receptors as possible drug targets for diabetes treatment - Nature Medicine 2015 (Marquard et al., 2015)
  Original research article, * equally contributed

- J. Marquard, A. Stirban, F. Schliess, F. Sievers, A. Welters, **S. Otter**, A. Fischer, S. Wnendt, T. Meissner, T. Heise, E. Lammert - Effects of dextromethorphan as add-on to sitagliptin on blood glucose and serum insulin concentrations in individuals with type 2 diabetes mellitus: a randomized, placebo-controlled, double-blinded, multiple crossover, single-dose clinical trial - Diabetes, Obesity and Metabolism 2016 (Marquard et al., 2016)
  Original research article

- **S. Otter**, E. Lammert - Exciting Times for Pancreatic Islets: Glutamate Signaling in Endocrine Cells - Trends in Endocrinology & Metabolism 2016 (Otter and Lammert, 2016)
  Review article

After a general introduction, a more specific introduction is provided to put the publications into their respective scientific context. With regard to the content, the publications are listed in a non-chronological order starting with the article Marquard et al., 2015, followed by Marquard et al., 2016 and the review article Otter and Lammert, 2016. The last article that is presented is Marquard et al., 2013. A summary of each article is provided. At the end of the thesis, concluding remarks with an outlook are included to further highlight the most important aspects of this work.

Please note that this thesis has continuous page numbers, although the page numbers are not shown on the respective manuscript pages.

2 Introduction

2.1 Pancreatic islets - the hormone productive site of the pancreas

The pancreas is divided into an exocrine part and an endocrine part, both having different physiological functions. The exocrine tissue is a branching network of ducts composed of acinar, centroacinar and duct cells and secretes digestive enzymes and a bicarbonate-rich fluid into the small intestine (Cleveland et al., 2012; Low et al., 2010). The endocrine part of the pancreas is called pancreatic islets or islets of Langerhans and is responsible for the production and secretion of various peptide hormones that are key regulators of blood glucose control (glucose homeostasis) (Caicedo, 2013). Comprising 50-3,000 cells, pancreatic islets are individual cell clusters with a diameter of 50-500 µm that are embedded in the exocrine tissue and are scattered throughout the whole pancreas (Rutter et al., 2015). With a number of around 1 million islets per human pancreas the endocrine part makes up 1-2% of the whole pancreatic tissue volume (Rorsman and Braun, 2013). Islets are composed of different endocrine cell types. Whereas the number of insulin-producing beta cells predominates, islets further consist of alpha, delta, epsilon and pancreatic polypeptide (PP) cells which produce and secrete the hormones glucagon, somatostatin, ghrelin and PP respectively (Eberhard et al., 2008; Folias and Hebrok, 2014).

Pancreatic islets are highly vascularized by fenestrated capillaries that built up a dense capillary network in the islets (Eberhard et al., 2010). Despite the small contribution of the endocrine pancreas to the whole pancreatic tissue volume, this network enables the islets to receive approximately 10% of the total blood supply of the pancreas (Jansson and Hellerstrom, 1986). It has been shown that the dense vascularization of the islets is important for normal islet function and that the vascular endothelial growth factor A (VEGF-A) derived from islet cells is a key regulator for the development of this fenestrated capillary network (Eberhard et al., 2010; Lammert et al., 2003).

Regarding the architecture of pancreatic islets, differences between species exist in particular between mouse and human (Cabrera et al., 2006). Mouse islets consist of around 75% of insulin producing beta cells. These cells form an inner core in murine islets that is surrounded by the other endocrine cell types (Brissova et al., 2005). Therefore, most of the beta cells in murine islets are adjacent to the same endocrine cell typ. In contrast, human islets show a diverse organization of the islet architecture: The different endocrine cell types are scattered throughout the whole islet, allowing a higher proportion of cell to cell contacts between the different endocrine cell types. Furthermore, human islets have a higher variability in the proportion of endocrine cell types than mouse islets which are more homogenous (Brissova et al., 2005; Cabrera et al., 2006). On average, human islets consist of around 55% of beta cells thus showing a smaller amount of beta cells than mouse islets, whereas the average percentage of alpha cells is higher in human compared to murine islets (38% versus 18%) (Cabrera et al., 2006).

Furthermore, the innervation pattern differs between mouse and human pancreatic islets. Mouse islets show strong innervation seen as direct contacts between pancreatic endocrine cells and nerve terminals. In human islets, direct contacts of these cell types are rarely seen. Instead smooth muscle cells of blood vessels are innervated by nerve terminals in human islets (Rodriguez-Diaz et al.,

2011a). The presence of smooth muscle cells throughout the whole vascular network is another distinctive feature between mouse and human islets, because in mouse islets blood vessels have been found to be less often associated with smooth muscle cells (Rodriguez-Diaz et al., 2011a). It has been proposed that the differences in the islet architecture between mouse and humans are associated with physiological differences regarding islet function (Cabrera et al., 2006).
In sum, pancreatic islets are kind of mini-organs distributed throughout the whole pancreatic tissue, that play a key role in the regulation the body's glucose homeostasis by producing and appropriately secreting different pancreatic peptide hormones into the blood stream.

2.2 Regulation of insulin secretion and glucose homeostasis

Glucose is one of the most important energy sources for the body as the brain almost completely depends on the provision of glucose as energy substrate (Mergenthaler et al., 2013). The maintenance of glucose homeostasis is important to assure the permanent supply of adequate amounts of glucose as both, too low (hypoglycemia) and elevated (hyperglycemia) blood glucose concentrations are associated with serious acute and long-term complications (Folias and Hebrok, 2014). Thus, independent of the food supply, the body's blood glucose concentration is always kept within a narrow range. This tight blood glucose control is mainly achieved by the bihormonal control of insulin and glucagon, secreted by beta and alpha cells respectively, and is further influenced by other hormones. Due to their opposing actions on blood glucose concentration, insulin and glucagon are two counterregulatory hormones. Insulin action mediates blood glucose lowering effects. In contrast, when blood glucose concentration is low, glucagon release from pancreatic alpha cells is triggered. This leads to the stimulation of hepatic glucose output, which results in an increase of blood glucose concentration and thus assures that glucose homeostasis is maintained (Folias and Hebrok, 2014; Jiang and Zhang, 2003).
Insulin is stored in secretory granules in beta cells and upon stimulation these granules fuse with the plasma membrane in order to release insulin. Within the secretory granules, insulin molecules are associated with two Zn^{2+} ions to form hexameric-structures (Li, 2014). Electron microscopy revealed that single rat beta cells comprise around 5,000-6,000 insulin-containing secretory granules (Fava et al., 2012). The major stimulus for insulin secretion is glucose that enters beta cells via glucose transporters (Folias and Hebrok, 2014). In mouse beta cells the glucose transporter Glut2 is present, whereas in human beta cells the predominant types of glucose transporters are thought to be GLUT1 and GLUT3 (McCulloch et al., 2011).
The following simplified model describes glucose-stimulated insulin secretion (GSIS) from beta cells (also see Figure 1): After entering the beta cell, glucose is metabolized resulting in an increase of the cytosolic ATP/ADP ratio (Folias and Hebrok, 2014). Beta cells are equipped with ATP-sensitive K^+ channels (K_{ATP} channels) (Ashcroft and Rorsman, 1990). A high cytosolic ATP/ADP ratio triggers the closure of these channels ultimately leading to the depolarization of the plasma membrane. Finally, the activation of voltage-dependent Ca^{2+} channels (VDCCs) results in an influx of Ca^{2+} ions which is the ultimate signal for the secretory granules to fuse with the plasma membrane and to release insulin (Folias and Hebrok, 2014). In addition to the entry of Ca^{2+} ions through VDCCs, it has been shown

that Ca^{2+} released from intracellular Ca^{2+} stores for example the endoplasmic reticulum (ER) contributes to the rise of cytosolic Ca^{2+} concentration and thus insulin release (Yang et al., 2014). Various SNARE proteins that are associated with the plasma membrane of the beta cell or the membrane of the secretory granules play a critical role in the Ca^{2+}-dependent fusion of the insulin secretory granules with the plasma membrane (Gaisano, 2014; Rorsman and Braun, 2013).

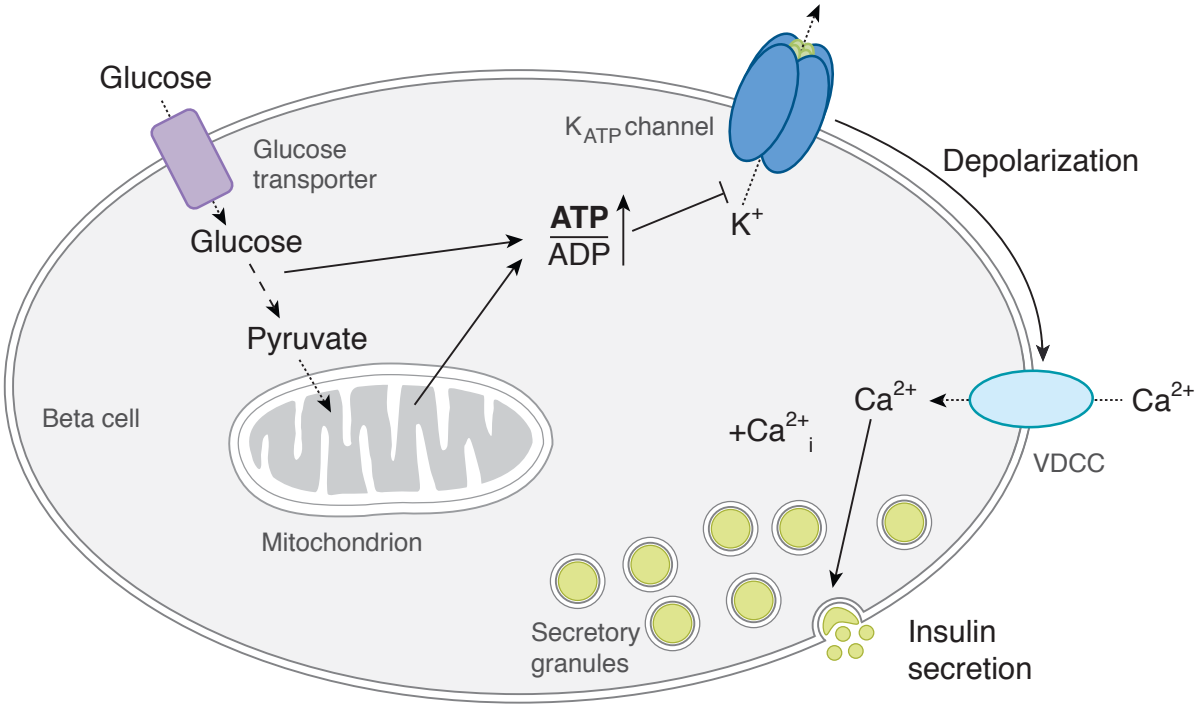

Figure 1. Model of glucose-stimulated insulin secretion (GSIS) from beta cells. Glucose metabolism increases the cytosolic ATP/ADP ratio that triggers the closure of K_{ATP} channels. The final resulting depolarization of the plasma membrane leads to Ca^{2+} influx through activated VDCCs. This Ca^{2+} influx together with Ca^{2+} released from intracellular stores results in the fusion of insulin-containing secretory granules with the plasma membrane and insulin secretion. For more detailed description see text. K_{ATP} channel, ATP-sensitive K^+ channel; VDCC, voltage-dependent Ca^{2+} channel; Ca^{2+}_i, Ca^{2+} released from intracellular stores. Dotted arrows: transported to; dashed arrows: metabolized to; solid arrows: lead to. This figure contains elements from Otter and Lammert, 2016.

Furthermore other channels such as Ca^{2+}-activated K^+ channels contribute to the regulation of GSIS from beta cells (Rorsman and Braun, 2013). Small conductance Ca^{2+}-activated K^+ channels 4 (SK4 channels) are present in human and mouse pancreatic islets and have been shown to be involved in the coupling of GSIS (Dufer et al., 2009; Rorsman and Braun, 2013).

In beta cells, the initial step of glucose metabolism, which is the phosphorylation of glucose, is catalyzed by glucokinase, an isoenzyme of the hexokinase. It has a lower affinity to glucose than other hexokinases and exhibits half-maximal activity at glucose concentrations in the millimolar range thus being a rate-limiting step in insulin secretion (Folias and Hebrok, 2014; Lenzen, 2014; Rorsman and Braun, 2013).

Furthermore, there is evidence that intracellular metabolic coupling factors such as glutamate, GTP and NADPH are produced by beta cells during glucose metabolism that contribute to appropriate insulin secretion (Maechler, 2013; Prentki et al., 2013). GSIS is characterized by a biphasic secretion pattern. The first-phase insulin secretion lasts five to ten minutes and during this period a high

amount of insulin is rapidly secreted (Rorsman and Renstrom, 2003). It has been suggested that this first-phase is associated with a distinct pool of insulin granules, the so-called readily releasable pool (RRP) (Ashcroft and Rorsman, 2012). The subsequent second-phase insulin secretion lasts longer but shows a smaller amount of secreted insulin per minute compared to first-phase secretion (Rorsman and Renstrom, 2003).

Insulin is secreted in a pulsatile fashion, which is considered to have important physiological functions with regards to the amount of insulin secreted and the effectiveness of insulin signaling in its target tissues (Satin et al., 2015). The molecular basis of the pulsatile fashion of insulin secretion are oscillations of the beta cell membrane potential and cytoplasmic Ca^{2+} concentration (Henquin, 2009).

Besides glucose, other nutrients such as amino acids and fatty acids can act as insulin secretagogues and lead to fuel-induced insulin secretion (Prentki et al., 2013). Additionally, insulin secretion from pancreatic beta cells can be influenced and modulated by other factors such as hormones and neurotransmitters that enhance or inhibit insulin secretion including autocrine signaling and paracrine interactions within the islet (Rorsman and Braun, 2013).

Glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are so-called incretin hormones. They are secreted by intestinal L- and K-cells respectively after a meal intake and have the ability to potentiate postprandial insulin secretion (Drucker and Nauck, 2006). The term “incretin effect” refers to the phenomenon that the insulin secretory response of the body is enhanced when glucose is administered orally compared to an isoglycemic intravenous administration (Elrick et al., 1964; Holst, 2007). Incretin receptors are expressed in pancreatic beta cells and several other tissues and organs such as heart and brain (Holst, 2007). GLP-1 receptor activation in beta cells leads to the potentiation of postprandial insulin secretion by increasing cAMP levels and activating downstream cAMP-dependent signaling pathways including PKA and Epac2 (Drucker, 2006). Besides, more recent data indicate that cytosolic glutamate is involved in the signaling pathway leading to incretin-stimulated insulin secretion (Gheni et al., 2014).

Several studies have shown that the excitatory neurotransmitter glutamate can modulate insulin secretion (Bertrand et al., 1995; Feldmann et al., 2011; Gheni et al., 2014; Maechler and Wollheim, 1999). In Otter and Lammert, 2016 the role of intra- and extracellular glutamate in the endocrine pancreas regarding insulin secretion and islet function is summarized and discussed (see section 4.3). Besides glutamate, other neurotransmitters such as GABA and acetylcholine play a regulatory role in insulin secretion and probably in the secretion of other pancreatic hormones (Braun et al., 2010; Koh et al., 2012; Molina et al., 2014). Interestingly, the source of acetylcholine differs between mouse and human pancreatic islets: While in mouse pancreatic islets, acetylcholine is released by nerve terminals, in human pancreatic islets it is released by alpha cells (Rodriguez-Diaz et al., 2011b).

Additionally, hormones released by the other endocrine cell types of the pancreas further influence insulin secretion from beta cells. For example somatostatin released by delta cells is a negative regulator of insulin secretion. Activation of somatostatin receptors on beta cells inhibits insulin secretion due to hyperpolarization of the beta cell plasma membrane and has a direct inhibitory effect on granule exocytosis (Kailey et al., 2012; Rorsman and Braun, 2013).

To sum up, beta cells act as fuel-sensors due to their ability to couple the body's nutrient state with changes in membrane potential and cytosolic Ca^{2+} concentration in order to regulate insulin secretion. Adequate insulin secretion from pancreatic beta cells is a result of a well-controlled interplay between the ability of beta cells to sense nutrients and to receive external regulatory signals including paracrine and autocrine signaling.

2.3 Action of insulin

Insulin mediates its action primarily by binding to insulin receptors. The insulin receptor is a receptor tyrosine kinase and assembles as tetramer but can also form a functional hybrid receptor with the highly related insulin-like growth factor receptor 1 (Bedinger and Adams, 2015; Taniguchi et al., 2006). The classical insulin-sensitive tissues are the liver, adipose tissue and skeletal muscle. However, insulin receptors are widely expressed in most of the tissues in the body (Bruning et al., 2000). Activation of the insulin receptor leads to the phosphorylation of insulin receptor substrate proteins, which is linked with the activation of two major signaling pathways: PI3K/Akt and Ras/MAPK signaling pathway. The PI3K/Akt signaling pathway is involved in most of the metabolic actions of insulin, whereas the Ras/MAPK pathway is primarily responsible for cell growth-related gene expression (Siddle, 2011; Taniguchi et al., 2006). Therefore, insulin has many biological functions. The primary metabolic action of insulin is the stimulation of the uptake of glucose, free fatty acids and amino acids into cells and thus the reduction of their respective concentrations in the blood. Furthermore, insulin promotes the intracellular storage of these nutrients (Folias and Hebrok, 2014; Saltiel and Kahn, 2001). By binding to the insulin receptor located on skeletal muscle and adipose tissue, insulin stimulates the translocation of the glucose transporter GLUT4 to the plasma membrane and thus promotes glucose uptake from the blood into these tissues (Saltiel and Kahn, 2001). Besides, insulin has an impact on the carbohydrate metabolism of the liver as it stimulates glycogen synthesis (glycogenesis) and inhibits its breakdown (glycogenolysis) in the liver and skeletal muscle. Furthermore, endogenous glucose production due to gluconeogenesis is suppressed in the liver by insulin action (Dimitriadis et al., 2011; Folias and Hebrok, 2014; Saltiel and Kahn, 2001).

Regarding the body's lipid metabolism, insulin promotes the synthesis of fatty acids (lipogenesis) in adipose tissue and the liver and further increases triglyceride synthesis. Simultaneously it suppresses fatty acid oxidation and triglyceride breakdown (Holt and Hanley, 2007). In addition, insulin increases the uptake of amino acids from the blood and promotes protein synthesis in several tissues including skeletal muscle and liver and it inhibits protein breakdown (Dimitriadis et al., 2011).

Besides acting on the classical insulin-sensitive tissues such as muscle, liver and adipose tissue, insulin additionally was found to act on the brain by binding to insulin receptors that are widely expressed in the brain. Insulin action in the brain is not only thought to be involved in energy homeostasis, it is also thought to play an important role in reproductive endocrinology and neuronal survival (Plum et al., 2005).

Beta cells express insulin receptors and there is evidence that insulin acts as autocrine signal, although it is debated whether it functions as stimulatory or inhibitory autocrine signal for insulin secretion (Tengholm and Gylfe, 2009). Additionally, the view that insulin itself serves as autocrine

signal for beta cells to trigger the insulin signaling pathway has been questioned (Rhodes et al., 2013).

In sum, insulin is a hormone with multiple biological functions. It plays a pivotal role in glucose homeostasis by mediating blood glucose lowering effects due to various mechanisms. Furthermore, it has an anabolic function and is involved in cell growth.

2.4 Diabetes mellitus - a metabolic and insulin secretion disorder

Diabetes mellitus is a complex metabolic and insulin secretion disorder that is characterized by high blood glucose concentrations resulting from the insufficient supply of insulin for the body's needs (Ashcroft and Rorsman, 2012). Diabetes has reached pandemic proportions as today, there are almost 350 million people affected worldwide and estimations predict that in 2030 around 500 million people will suffer from this disease (Vetere et al., 2014). Diabetes is associated with various long-term complications affecting the neuronal and cardiovascular system, including both macrovascular and microvascular events such as stroke, myocardial infarction, nephropathy and retinopathy (Ashcroft and Rorsman, 2012; Leon and Maddox, 2015). Additionally, recent studies revealed that diabetes is associated with an increased risk of some cancers such as liver, kidney and pancreatic cancer (Harding et al., 2015). This makes diabetes a leading cause of death worldwide and also to an enormous economic burden for the healthcare systems (Ashcroft and Rorsman, 2012; WHO, 2008).

Diabetes mellitus can be broadly classified into two main types: Type 1 diabetes mellitus (T1D) accounts for approximately 5% of all cases, whereas around 95% of all diabetes patients suffer from type 2 diabetes mellitus (T2D) (Vetere et al., 2014). Besides these two main types of diabetes other forms of diabetes exist including the monogenetic disorder maturity-onset diabetes of the young (MODY) and gestational diabetes (ADA, 2013). MODY is a rare form of diabetes and is caused by mutations in genes involved in beta cell function (ADA, 2013; Thanabalasingham and Owen, 2011). Gestational diabetes can occur during pregnancy and is associated with an increased risk of developing diabetes mellitus for mother and child after delivery (ADA, 2004).

T1D is an autoimmune disease that is characterized by the progressive loss of insulin-secreting beta cells and the infiltration of immune cells into pancreatic islets plays a pivotal role in this process. T1D primarily but not exclusively affects children and adolescents (Atkinson et al., 2014). The course of this disease starts with a presymptomatic period that varies in its length from months to decades (Insel et al., 2015). During this presymptomatic period, islet autoimmunity takes place leading to the progressive destruction of insulin-secreting beta cells. Islet autoimmunity can be detected through the presence of autoantibodies against beta cell autoantigens such as insulin, glutamic acid decarboxylase or zinc transporter 8 (Atkinson et al., 2014). Ultimately, when a critical number of around 80-95% of beta cells is destroyed, the insulin supply is insufficient for the body's needs resulting in hyperglycemia and thus overt T1D (Welters and Lammert, 2014). In approximately 60% of all cases, a temporary phase of partial remission the so-called honeymoon phase takes place shortly after clinical diagnosis and begin of the treatment. This honeymoon phase is characterized by restored endogenous insulin release and typically lasts three to six months, but the duration may continue for two years (van Belle et al., 2011).

Both, genetic predisposition and environmental factors play an important role in the pathogenesis of T1D. First- and second-degree relatives from type 1 diabetics have a 10- to 20-fold increased risk to develop T1D compared to individuals that do not have close relatives with T1D (Insel et al., 2015). T1D is classified as polygenetic disorder, as multiple loci have been identified to affect the risk of developing T1D. Many of these genes are located in the human leukocyte antigen (HLA) region on chromosome 6 encoding for genes involved in immune responses (Atkinson et al., 2014; Noble et al., 2010).

In connection with the contribution of environmental factors to the development of T1D, several candidates have been proposed such as viruses, diets and vitamin D pathway components and it has been suggested that the environmental influence can start as early as in the utero (Atkinson et al., 2014).

T2D is referred to as adult-onset diabetes (ADA, 2013). However, the prevalence of T2D in young individuals is increasing (Cameron and Wherrett, 2015; Tuomi et al., 2014). The risk to develop T2D is influenced by age and lifestyle factors such as physical inactivity and obesity. In addition, genetic predisposition has a high impact on the risk to develop this disease (ADA, 2013; Ashcroft and Rorsman, 2012). The main hallmarks of T2D are insulin resistance and the progressive loss in beta cell function (Tahrani et al., 2011). Insulin resistance is the decreased ability to appropriately respond to insulin released by pancreatic beta cells, that can affect several tissues and organs including skeletal muscle, liver and adipose tissue (Goldstein, 2002).

It is commonly accepted that obesity can promote insulin resistance and that multiple factors are involved in this process: Excessive obesity causes a metabolic overload of the adipose tissue resulting in adipocyte enlargement. Further overloading of the enlarged adipocytes finally triggers the secretion of inflammatory cytokines such as monocyte chemoattractant protein-1 (MCP-1) and tumor necrosis factor alpha (TNF-alpha) from adipocytes (Guilherme et al., 2008). Chronic inflammation of the adipose tissue is further supported by the infiltration of immune cells such as macrophages that contribute to the amount of inflammatory cytokines released in the adipose tissue. This eventually leads to enhanced levels of non-esterified fatty acids (NEFAs) and glycerol in the blood due to increased lipolysis (Guilherme et al., 2008; Welters and Lammert, 2014). An increase of NEFA concentrations in the blood is thought to be the most pivotal factor linking obesity to the development of insulin resistance (Tahrani et al., 2010). In addition, the imbalance of hormones secreted by adipocytes (adipokines) such as leptin and adiponectin play an important role in the development of insulin resistance (Tahrani et al., 2010; Welters and Lammert, 2014).

In insulin-resistant skeletal muscles, insulin-induced glucose uptake from the blood is reduced due to impaired translocation of GLUT4 to the plasma membrane (Karlsson and Zierath, 2007). Hepatic insulin resistance leads to a diminished suppression of hepatic glucose output, which is due to reduced suppression of gluconeogenesis and reduction of glycogen synthesis (Perry et al., 2014). It is known that there are fluctuations in insulin sensitivity during life time, for example the occurrence of insulin resistance during puberty or pregnancy (Kahn et al., 2006).

Importantly, insulin resistance alone does not necessarily lead to the development of T2D. Most of the insulin-resistant obese individuals do not suffer from T2D (Tahrani et al., 2010). This is due to the

fact that pancreatic beta cells can often compensate for the diminished action of insulin to maintain glucose homeostasis by increasing the amount of released insulin up to fourfold compared to lean persons (Cavaghan et al., 2000). Another possible mechanism to compensate for insulin resistance is the expansion of beta cell mass, which has been observed in obese non-diabetic individuals (Ferrannini, 2010). Insulin resistance and thus upregulation of beta cell function often precedes an overt T2D for many years. The critical event that drives the further development of T2D is the decline in beta cell function resulting in the inability of pancreatic beta cells to further compensate for the body's increased demand of insulin (Tahrani et al., 2010). Various factors exist that are thought to contribute to beta cell dysfunction and may ultimately lead to beta cell death including chronic hyperglycemia (glucotoxicity), chronic exposure to NEFAs (lipotoxicity) and genetic factors (Tahrani et al., 2011; Welters and Lammert, 2014). Regarding the contribution of genetic factors, to date more than 80 loci have been identified that are associated with an increased risk of developing T2D (Grarup et al., 2014; Wang et al., 2016). Among these, *TCF7L2* is the strongest diabetes susceptibility locus and is associated with beta cell dysfunction (Grarup et al., 2014; Nolan et al., 2011).

The contribution of the decline of beta cell mass to the pathophysiology of T2D has long been debated (Donath et al., 2003). However, postmortem analysis of human pancreases from non-diabetic and type 2 diabetic donors revealed that the relative beta cell volume was significantly decreased in those individuals with T2D compared to the respective controls (Butler et al., 2003). There is evidence that apoptosis is an important mediator of beta cell mass decline occurring in T2D (Ardestani et al., 2014; Butler et al., 2003; Rhodes, 2005). Recently, this view has been challenged by a research group proposing that beta cell dedifferentiation into non-beta cells is the major contributor to the progressive loss of beta cell mass in T2D (Cinti et al., 2015; Talchai et al., 2012). However, this has been debated (Butler et al., 2016).

In addition to beta cell dysfunction, individuals with T2D often exhibit abnormal glucagon secretion pattern with increased fasting glucagon concentrations and reduced suppression of postprandial glucagon secretion. Because glucagon stimulates hepatic glucose output, alpha cell dysfunction contributes to both an increase of fasting as well as postprandial glucose concentrations in type 2 diabetics (Del Prato and Marchetti, 2004).

In sum, diabetes mellitus is a heterogeneous disease that is characterized by elevated blood glucose concentrations and progressive beta cell loss, and both, lifestyle as well as genetic factors play an important role in the development of this disease.

2.5 Strategies for the treatment of diabetes mellitus

The primary aim of the treatment of diabetes mellitus is the maintenance of blood glucose concentrations within or near to the physiological range (Welters and Lammert, 2014). Due to differences in the pathophysiology of T1D and T2D, different strategies exist to achieve this aim. T1D is characterized by the destruction of most of the functional beta cell mass. This destruction usually results in an absolute insulin deficiency, thus leading to the dependency of individuals suffering from T1D on exogenous administration of insulin (ADA, 2013; Welters and Lammert, 2014). Some

individuals with T1D still have the capability to typically secrete low amounts of endogenous insulin. Although the amount of endogenously secreted insulin is not sufficient for the body's needs of insulin, it is important to maintain this endogenous insulin secretion, as it is associated with less long-term complication (Atkinson et al., 2014).

Nowadays, insulin analogues with diverse duration of biological activity and different ways of application exist to give space for individualized insulin therapy. Intensive insulin therapy includes multiple injections per day with long-acting insulin mimicking basal insulin secretion and rapid-acting insulin that is administrated directly before food intake (Atkinson et al., 2014). Strong efforts are undertaken to further develop insulin therapy in order to improve the quality of life and reduce the risk of hypoglycemia. One example is the development of a closed loop system that acts as an artificial pancreas by combining an insulin pump and a continuous glucose monitor with a computer algorithm (Breton et al., 2012; Cameron and Wherrett, 2015).

Pancreatic islet transplantation represents another approach to treat T1D. Due to its associated risks, this procedure is reserved for selected adult individuals with T1D that suffer from hypoglycemia and have poor glycemic control (McCall and Shapiro, 2014). In the last years, significant progress was made regarding the process of islet transplantation (Farney et al., 2016). However, long-term studies revealed that five years after transplantation only around 10% of the patients who underwent islet transplantation were still independent of exogenous insulin administration (Ryan et al., 2005). Thus, until now, islet transplantation remains a rather experimental procedure and more research has to be done to improve efficiency of this approach (Atkinson et al., 2014).

Complementing the treatment of symptomatic T1D and taking into account that T1D has become a predictable disease, great effort is currently put into studies to investigate how the onset of T1D can be delayed or even prevented. These so-called prevention studies make use of several approaches including antigen-based therapy and special diets for children and infants (Atkinson et al., 2014; Beyerlein et al., 2014; Knip et al., 2014; Nanto-Salonen et al., 2008).

In contrast to the limited therapy approaches for the treatment of T1D, strategies for the treatment of T2D show a wider variety. Today, there are multiple drugs available for the treatment of T2D which differ in their drug targets and result in different modes of action (Kahn et al., 2014).

Due to the progressive nature of the disease, the treatment of T2D generally features a stepwise approach. The initial therapy often consists of lifestyle interventions such as weight reduction and physical activity. Usually metformin is the first antidiabetic drug that is prescribed when lifestyle interventions fail to meet the therapeutic goal which is the maintenance of glycated hemoglobin (HbA1c) below 7.0% (Nathan et al., 2009). Metformin belongs to the class of biguanides and its major mode of action is the suppression of hepatic glucose output and improvement of insulin sensitivity (Tahrani et al., 2010). When the disease proceeds, monotherapy is often not sufficient to maintain blood glucose concentrations in the target range and patients require additional medications, which leads to a combination of two or more antidiabetic drugs (Nathan et al., 2009). Other oral antidiabetic drugs that are currently available are sulfonylureas, a class of drugs that increase insulin secretion by binding to the SUR1 subunit of the K_{ATP} channel on pancreatic beta cells and thiazolidinediones that belong to the class of so-called insulin sensitizers which improve the insulin sensitivity of muscle, fat

and liver (Nathan et al., 2009; Tahrani et al., 2011). Alpha-glucosidase inhibitors represent another class of oral antidiabetic drugs that can promote the reduction of blood glucose concentrations by delaying the degradation of complex intestinal carbohydrates and thus resulting in retarded glucose absorption (Nathan et al., 2009).

Incretin-based therapies for the treatment of T2D include the oral administration of dipeptidyl peptidase-4 (DPP4) inhibitors and the injection of GLP-1 analogues that can activate GLP-1 receptors (Tahrani et al., 2011). As due to its rapid degradation resulting from the action of DPP4, endogenous GLP-1 has a short half-life of less than 2 minutes. Inhibition of DPP4 increases the endogenous GLP-1 concentration (Baggio and Drucker, 2007). Besides its increasing action on GSIS from pancreatic beta cells, GLP-1 receptor activation due to GLP-1 analogues also suppresses glucagon secretion and delays gastric emptying thus having multiple modes of antidiabetic actions (Tahrani et al., 2011). Recently, Na^+-glucose co-transporter 2 (SGLT2) inhibitors, a new class of oral antidiabetic drugs, have been introduced to the market. SGLT2 inhibitors act on the kidney by inhibiting the reabsorption of glucose from the urine in the proximal tubule due to action of SGLT2, and therefore have blood glucose reducing effects (Kahn et al., 2014; Liu et al., 2012). Often, due to the progressive nature of T2D the antidiabetic drugs mentioned above cannot maintain glycemic control and an intensive insulin therapy becomes necessary in order to meet the therapeutic goals of patients with T2D (Nathan et al., 2009).

All antidiabetic medications have their individual advantages and disadvantages. Insulin and sulfonylureas for example can lead to life-threatening hypoglycemic events and weight gain. Other drugs such as metformin, alpha-glucosidase inhibitors and GLP-1 analogues can trigger gastrointestinal side effects and thiazolidinediones are associated with increased risk of heart failure, fractures and edema (Tahrani et al., 2011). Furthermore, SGLT2 inhibitors are associated with an increased risk of mycotic genitourinary infections and urinary tract infections (Nathan, 2015). Unknown long-term safety profiles represent a disadvantage of the newer agents such as thiazolidinediones, GLP-1 analogues, DPP4 inhibitors and SGLT2 inhibitors compared to antidiabetic drugs that have been on the market for several decades such as sulfonylureas and metformin (Kahn et al., 2014; Tahrani et al., 2011). Additionally, GLP-1 analogues have unconfirmedly been associated with pancreatitis (Tahrani et al., 2010). However, animal experiments suggest, that incretin-based antidiabetic drugs may have beneficial effects on beta cell survival (Tahrani et al., 2011).

Based on the fact that diabetes increases the risk of cardiovascular diseases (Duca et al., 2013), antidiabetic drugs with high cardiovascular safety or even beneficial effects regarding the cardiovascular system are of great favor. Furthermore, until now antidiabetic drugs focus on the maintenance of glycemic control and currently no drug is available that has proven durable beta cell protective effects in humans. Such a drug would be beneficial for the treatment of both T1D and T2D, as both types are characterized by the decline of functional beta cell mass.

In sum, today there are good options to treat diabetes and possibilities to adapt the treatment to the individual needs of the patient (Nathan, 2015). However, until today, there are no drugs available that can stop the progression of the disease or even have curative effects and diabetes treatment therefore comes along with medication that must continue during the whole life span.

2.6 Hyperinsulinemic hypoglycemia - an insulin secretion disorder

Hyperinsulinemic hypoglycemia is characterized by a dysregulation of insulin secretion from pancreatic beta cells leading to inappropriate insulin secretion in spite of low blood glucose concentrations (Guemes and Hussain, 2015). As glucose is the primary fuel for the brain, inappropriate insulin secretion at low blood glucose concentrations endangers the fuel supply, which can lead to severe brain injury (Rozenkova et al., 2015). Hyperinsulinemic hypoglycemia usually occurs during the neonatal period but can also develop later in life during infancy or childhood (Guemes and Hussain, 2015).

Hyperinsulinemic hypoglycemia can have several causes. The most severe form is the inherited congenital hyperinsulinism. However, hyperinsulinemic hypoglycemia can also be induced by an insulinoma or can be transient when caused by other reasons including maternal diabetes mellitus or intra-uterine growth restriction (Rozenkova et al., 2015). The congenital hyperinsulinism can be divided into different histological subgroups. The diffuse form is characterized by dysfunctional beta cells located in the whole pancreatic tissue. The focal form does not affect the whole pancreas but is rather restricted to a specific area. These areas can occur in any region of the pancreas. In addition, an atypical form has been described which is characterized by enlarged beta cell nuclei (Rozenkova et al., 2015). To date, mutations in nine genes that are involved in the regulation of insulin secretion from pancreatic beta cells including *ABCC8*, *KCNJ11*, *GLUD1*, *GCK* and *MCT1* are associated with the congenital form of hyperinsulinism (Rozenkova et al., 2015). Mutations in *ABCC8* and *KCNJ11* that encode the two subunits of the K_{ATP} channel SUR1 and Kir6.2 respectively, are the most frequent cause of congenital hyperinsulinism (Kapoor et al., 2013). The mutations in *ABCC8* and *KCNJ11* can cause unregulated insulin secretion by several molecular mechanisms for example by affecting channel trafficking, channel biosynthesis or channel regulation (Rahman et al., 2015). Moreover, gain of function mutations of *GLUD1* encoding the mitochondrial enzyme glutamate dehydrogenase, are associated with inappropriate secretion of insulin upon fasting periods and protein meal intake and are further associated with increased plasma ammonia concentrations. Glutamate dehydrogenase catalyzes the oxidative deamination of glutamate to give rise to alpha-ketoglutarate and ammonia and vice versa in a NADP(H)- or NAD(H)-dependent fashion (Chandran et al., 2014; Plaitakis et al., 2013).

Since hyperinsulinemic hypoglycemia is a heterologous disease, treatment strategies comprise medical, dietary as well as surgical approaches with the primary objective to avoid hypoglycemic events that can lead to severe brain injury (Rozenkova et al., 2015).

3 Scientific context of the publications

3.1 Marquard et al., 2015 Nature Medicine; Marquard et al., 2016 Diabetes, Obesity and Metabolism; and Otter and Lammert, 2016 Trends in Endocrinology and Metabolism

In the past, many studies have revealed that pancreatic endocrine cells and neurons exhibit numerous similarities including the expression of neuronal cell adhesion molecules, the release of neurotransmitters such as glutamate, GABA and acetylcholine and the expression of specific receptors for these neurotransmitters (Arntfield and van der Kooy, 2011; Braun et al., 2010; Di Cairano et al., 2015; Feldmann et al., 2011; Koh et al., 2012; Rodriguez-Diaz et al., 2011b). Interestingly, the genome-wide mRNA profile of pancreatic endocrine cells is more similar to neuronal tissues than to other tissues with a common endoderm origin such as acinar cells or the liver (van Arensbergen et al., 2010).

Glutamate is an excitatory neurotransmitter with pivotal roles in the central nervous system (Hertz, 2006) and it is known that pancreatic islets express glutamate receptors and glutamate transporters necessary for glutamate-mediated signaling (Di Cairano et al., 2011; Feldmann et al., 2011; Inagaki et al., 1995; Molnar et al., 1995; Moriyama and Hayashi, 2003; Weaver et al., 1996). Several studies from different groups have shown that beta cells can form glutamate via their own glucose metabolism and that glutamate influences islet function both intra- and extracellularly (Cabrera et al., 2008; Di Cairano et al., 2011; Gheni et al., 2014; Maechler and Wollheim, 1999; Vetterli et al., 2012). Extracellular glutamate mediates its signaling by binding to two different classes of glutamate receptors: Ionotropic and metabotropic receptors. Ionotropic glutamate receptors form ion channels and N-methyl-D-aspartate receptors (NMDARs), alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) and kainate receptors belong to this class of glutamate receptors. Metabotropic glutamate receptors are G-protein-coupled receptors (Kalia et al., 2008).

In the past, progress in the investigation of the role of extracellular glutamate in islet function, especially hormone secretion from pancreatic islets, has been made. Glutamate has been found to mediate a positive feedback loop for glucagon secretion mediated by AMPARs and kainate receptors expressed on alpha cells (Cabrera et al., 2008). However, the investigation of the role of NMDARs in pancreatic islets regarding insulin secretion had been neglected in the past. NMDARs assemble as tetramers from different subunit classes: GluN1, GluN2 (GluN2A-GluN2D) and GluN3 (GluN3A-GluN3B). The GluN1 subunit is obligatory to form functional receptors (Traynelis et al., 2010). NMDARs are widely expressed throughout the central nervous system and are mainly located on postsynaptic neurons, but are also found on presynaptic neurons (Paoletti et al., 2013). Activation of NMDARs requires the binding of glutamate and glycine or D-serine as a co-agonist, and further requires the depolarization of the plasma membrane to release the Mg^{2+} block from the channel pore. Thus, in contrast to AMPARs and kainate receptors, NMDARs are both ligand- and voltage-gated (Paoletti et al., 2013). NMDARs are ion channels that are permeable for Ca^{2+}, Na^{+} and K^{+} ions (Kalia et al., 2008). In the central nervous system, activation of NMDARs can have many different physiologic functions. NMDARs mediate excitatory synaptic transmission and also play an important

role in synaptic plasticity thought to underlie memory and learning (Paoletti et al., 2013). Furthermore, overactivation of NMDARs by excessive amounts of glutamate can lead to neuronal injury or cell death, a process also known as excitotoxicity (Kalia et al., 2008). Glutamate-induced excitotoxicity is involved in several neurological disorders such as stroke and neurodegenerative diseases including Alzheimer's disease and Parkinson's disease. Because NMDARs are involved in diverse neurological disorders, they serve as drug targets for the treatment of many of these diseases (Kalia et al., 2008; Paoletti et al., 2013).

In contrast to the central nervous system, the role of NMDARs in pancreatic islets remained controversial, as little and inconsistent results regarding the role of NMDARs in insulin secretion and blood glucose control have been published. For example, treatment of isolated islets and cells of an insulin-secreting cell line with the NMDAR agonist NMDA were not conclusive as both, no and increasing effects on insulin secretion have been reported (Gonoi et al., 1994; Inagaki et al., 1995; Molnar et al., 1995). Furthermore, existing studies resulted in contradictory outcomes regarding the effect of NMDAR antagonists on insulin secretion and blood glucose control (Garrino and Henquin, 1987; Konrad et al., 2000; Lechin et al., 2009).

Therefore, in Marquard et al., 2015 we aimed to investigate the role of NMDARs in pancreatic islets in terms of islet function and islet cell survival. Using genetic as well as pharmacological experimental approaches combined with a small clinical study with type 2 diabetics, we were able to shed light on this contradictory issue as our data indicate that NMDARs are negative regulators of GSIS and might serve as possible drug targets for adjunct treatment of diabetes (Marquard et al., 2015).

The outcome of the clinical study revealed that dextromethorphan (DXM), an NMDAR antagonist and ingredient of many over-the-counter cough suppressants (Dicpinigaitis, 2015), improved the glucose tolerance and increased serum insulin concentrations in type 2 diabetics during a glucose tolerance test (Marquard et al., 2015). Furthermore, our findings including our *in vitro* results that a NMDAR antagonist in combination with a GLP-1 analogue showed an additive effect on GSIS from isolated pancreatic islets paved the way for a second clinical trial to further investigate NMDARs as possible drug targets for adjunct diabetes therapy. Therefore, in Marquard et al., 2016, the additive effect of DXM and sitagliptin, a DPP-4 inhibitor that is in clinical use for diabetes treatment (Tahrani et al., 2011), was explored in a small clinical study with type 2 diabetics.

Finally, given the great progress in the investigation of intra- and extracellular glutamate signaling in pancreatic islets that has been made in the recent years, Otter and Lammert, 2016 represents a comprehensive review about glutamate signaling in the endocrine pancreas, which summarizes and discusses the findings of the recent years.

3.2 Marquard et al., 2013 Diabetologia

Exercise-induced hyperinsulinism is a rare form of congenital hyperinsulinism, which is characterized by inappropriate insulin secretion following anaerobic exercise or experimental pyruvate load. Activating mutations in the promoter of the *MCT1* gene (also known as *SLC16A1*) are associated with this form of hyperinsulinism (Guemes and Hussain, 2015; Otonkoski et al., 2007; Pullen et al., 2012). During anaerobic exercise, the skeletal muscle produces lactate that is subsequently released into the blood leading to increased blood lactate concentrations. High concentrations of lactate correlate with high concentrations of pyruvate in the blood during anaerobic exercise (Otonkoski et al., 2003). *MCT1* encodes the monocarboxylate transporter 1 (MCT1) that transports monocarboxylates such as pyruvate and lactate across the plasma membrane in a proton-linked fashion (Halestrap and Price, 1999). Under physiological conditions *MCT1* expression and MCT1 protein levels are very low in pancreatic islets and purified beta cells (Otonkoski et al., 2007; Zhao et al., 2001). In contrast, it has been proposed that in beta cells, an activating mutation in the promoter of *MCT1* increases the levels of MCT1 protein leading to pyruvate uptake by these cells and subsequently to increased ATP production by pyruvate metabolism that can trigger insulin secretion. Thus, under anaerobic exercise conditions, when pyruvate concentration in the blood is high, an activating mutation in the *MCT1* gene would lead to inappropriate insulin secretion despite of low blood glucose concentrations (Otonkoski et al., 2007). This hypothesis was tested in mice with a beta cell specific overexpression of *Mct1* (Pullen et al., 2012). Indeed, isolated islets of these mice showed an insulin response upon pyruvate stimulation, which was absent in control islets. Furthermore, plasma insulin concentrations increased after a pyruvate load in the transgenic mice showing that pyruvate could trigger insulin secretion (Pullen et al., 2012).

Although genetic linkage analysis and sequencing of two families with cases of exercise-induced hyperinsulinism have shown an association of mutations in the promoter of *MCT1* with exercise-induced hyperinsulinism (Otonkoski et al., 2007), the lack of pancreatic tissue of affected individuals prevented the confirmation of *MCT1* overexpression in pancreatic islets in the past (Pullen et al., 2012). In Marquard et al., 2013, we showed for the first time that *MCT1* is expressed and MCT1 protein is present in an insulinoma of a patient suffering from symptoms of exercise-induced hyperinsulinism.

4 Publications

4.1 Characterization of pancreatic NMDA receptors as possible drug targets for diabetes treatment (Marquard et al., 2015)

4.1.1 Summary

In this original research article we investigated pancreatic NMDARs in terms of insulin secretion, glucose tolerance and maintenance of islet cell function. Although it has been known for a long time that pancreatic islets and insulin-secreting cell lines express NMDARs their role regarding islet function and glucose control remained conflicting (Gonoi et al., 1994; Inagaki et al., 1995; Konrad et al., 2000; Lechin et al., 2009; Moriyama and Hayashi, 2003). Using a variety of different experimental approaches including isolated mouse and human pancreatic islets, several mouse models and a phase IIa clinical study with type 2 diabetics, our findings of Marquard et al., 2015 suggest that pancreatic NMDARs act as negative regulators of insulin secretion.

In detail, we could show that inhibition of NMDARs in pancreatic islets increased GSIS from mouse and human islets while leaving basal insulin secretion largely unaffected. Furthermore, we revealed that mice lacking pancreatic NMDARs had a better glucose tolerance compared to control animals. Administration of NMDAR antagonists such as DXM or its active metabolite dextrorphan (DXO) improved glucose tolerance of wild type mice without introducing hypoglycemia. In contrast, DXO could not further increase GSIS from islets isolated from mice lacking pancreatic NMDARs. DXO administration in these mice had no effect on blood glucose control during a glucose tolerance test. Additionally the combination of DXO and a GLP-1 analogue had an additive effect on GSIS from isolated mouse and human pancreatic islets.

In the present article we further shed light on the underlying molecular pathway and investigated how inhibition of pancreatic NMDARs leads to increased GSIS. As it is well-known that GSIS from pancreatic beta cells involves the closure of K_{ATP} channels, the depolarization of the plasma membrane and the entry of Ca^{2+} ions via VDCCs (Folias and Hebrok, 2014), we investigated whether inhibition of NMDARs influences this triggering pathway. Indeed, using a combination of genetic and pharmacological approaches we could show that the increasing effect of NMDAR inhibition on GSIS was dependent on the presence of functional K_{ATP} channels in pancreatic islets. We found that pharmacological inhibition of NMDARs prolonged the time that glucose-stimulated beta cells stayed in the depolarized phase, and using Ca^{2+} imaging techniques we showed that pharmacological inhibition of NMDARs additionally increased the plateau fractions of Ca^{2+} oscillations in glucose-stimulated beta cells and pancreatic islets. The increasing effect of NMDAR inhibition on GSIS from isolated pancreatic islets was absent when VDCCs were pharmacologically blocked and was weaker in islets lacking SK4 channels.

In addition, we investigated possible islet cell protective effects of NMDAR inhibition. Long-term treatment of *db*/*db* mice, a type 2 diabetic mouse model, with a high dose of DXM via the drinking water resulted in higher insulin content in isolated pancreatic islets and larger islet cell mass compared to control mice after eight weeks of treatment. Furthermore, the onset of diabetes was

delayed in *db*/*db* mice receiving a high dose of DXM compared to control mice as assessed by lower fasting blood glucose concentrations during the whole treatment time.

To further explore the potential beneficial effects of NMDAR antagonists on islet cell function and survival, we tested whether NMDAR antagonists could protect pancreatic islets from inflammatory cytokine-induced cell death. Indeed, we could show that treatment of isolated human islets from different donors with DXO resulted in less inflammatory cytokine-induced cell death *in vitro*.

Finally, our findings encouraged us to conduct a phase IIa clinical study to test whether administration of NMDAR antagonists also have blood glucose lowering effects in humans with T2D. Single dose administration of DXM enhanced serum insulin concentrations after a glucose challenge and improved glucose tolerance without introducing hypoglycemia in type 2 diabetics.

To sum up, in Marquard et al., 2015 we show that pancreatic NMDARs are involved in the regulation of GSIS and blood glucose control. Our findings indicate that pancreatic NMDARs are negative regulators of GSIS and suggest that pancreatic NMDARs are potential drug targets for adjunct treatment of diabetes.

4.1.2 Manuscript

See page 21 and following

Please note that the published version of this thesis does not contain the manuscript pages.

Reference:

Marquard, J., Otter, S., Welters, A., Stirban, A., Fischer, A., Eglinger, J., Herebian, D., Kletke, O., Klemen, M.S., Stozer, A., Wnendt, S., Piemonti, L., Kohler, M., Ferrer, J., Thorens, B., Schliess, F., Rupnik, M.S., Heise, T., Berggren, P.O., Klocker, N., Meissner, T., Mayatepek, E., Eberhard, D., Kragl, M., and Lammert, E. (2015). Characterization of pancreatic NMDA receptors as possible drug targets for diabetes treatment. Nature medicine 21, 363-372.
doi:10.1038/nm.3822.

Weblink:

http://www.nature.com/nm/journal/v21/n4/full/nm.3822.html

4.1.3 Personal contribution and general information

Personal contribution of Silke Otter (SO) to the manuscript “Characterization of pancreatic NMDA receptors as possible drug targets for diabetes treatment”

Name of the journal:	Nature medicine
Impact factor (2014):	27.363
Author position:	second author, equal contribution to first authorship
Tasks:	performance and analysis of experiments, planning of project, figure preparation, preparation and correction of manuscript, supplement and source data files, statistical analyses, coordination of collaborations

Author’s contribution adapted from the manuscript:

J.M. performed the initial experiments; J.M., A.W. and, to some extent, **S.O.** performed insulin secretion assays; J.M., **S.O.**, A.W. performed IPGTTs; J.M. carried out dextromethorphan treatment of *db/db* mice and performed experiments with INS1E cells; **S.O.** determined islet cell proliferation, beta cell mass and apoptosis, and performed the western blots and Ca2+ measurements set up by M. Köhler; A.W. performed *in vitro* islet cell viability experiments, reproduced the effects of dextromethorphan treatment in *db/db* mice and determined corticosterone concentrations; P.-O.B. introduced the idea of NMDAR-regulated Ca2+ oscillations to E.L., designed and performed the dynamic insulin release measurements, and together with M. Köhler established the technique for Ca2+ measurements in the laboratory of E.L.; M. Kragl and D.E. supervised J.M. and **S.O.** in standard techniques of islet biology and glucose measurements and helped with mouse experiments; J.E. trained **S.O.** and A.W. in image analysis and contributed to statistical analyses; A.F. drew a statistical analysis for clinical trial design and performed statistical analysis of human and mouse data; A. Stirban, F.S. and T.H. guided the clinical trial whose study protocol was written by A. Stirban with input from F.S., T.H., E.L., J.M. and T.M.; insulin and DXM concentrations from the clinical trial were measured by S.W. and D.H., respectively; J.F. and B.T. generated and analyzed the *Ins1-cre* mice; T.M. introduced the idea to use DXM for treatment of hyperinsulinism from islets to E.L.; M.S.K., A. Stožer and M.S.R. performed the Ca2+ and membrane potential measurements in pancreatic sections; O.K. and N.K. gave intellectual and experimental input into NMDAR physiology; E.M. co-supervised A.W.; L.P. performed human islet isolation; and E.L. conceptually designed most parts of this work, introduced the idea to use DXM as an antidiabetic compound, genetically study GluN1 as well as test DXM on islet cell viability and in GluN1 deficient mice, *db/db* mice and human individuals with T2DM, guided J.M., **S.O.** and A.W. in biweekly meetings, and wrote the article with them.

Contribution in detail:

Silke Otter performed the experiments for the following figures: Fig. 2d,f,g; Fig. 4e,f; Fig.5 d-j; Suppl. Fig 1a,b,g-i; Suppl. Fig 3a-e.

Silke Otter contributed to the experiments for the following figures: Fig. 3d,f; Fig. 5a,b; Suppl. Fig. 5c; Suppl. Fig. 6c,d.

Silke Otter was the leading coordinator for the collaborations resulting in the following figures: Fig. 2a,b,i,j; Suppl. Fig. 2g.

Silke Otter reproduced the results of the following figures to confirm data and to meet the journal's requirements for statistical analysis: Fig.1c; Fig. 2c; Fig. 3e; Suppl. Fig. 2c,d; Suppl. Fig. 4d.

In sum, all first authors Silke Otter, Jan Marquard and Alena Welters, contributed equally to the experimental work of this manuscript with approximately 28% each.

4.2 Effects of dextromethorphan as add-on to sitagliptin on blood glucose and serum insulin concentrations in individuals with type 2 diabetes mellitus: a randomized, placebo-controlled, double-blinded, multiple crossover, single-dose clinical trial (Marquard et al., 2016)

4.2.1 Summary

The clinical study presented in this article was a follow-up clinical study to Marquard et al., 2015 and a proof-of-concept study to investigate whether a low dose of the NMDAR antagonist DXM in combination with the DPP4 inhibitor sitagliptin can have additive effects on blood glucose and serum insulin concentrations during an oral glucose tolerance test. This clinical study was carried out with 20 individuals with T2D. Three different low doses of DXM 30 mg, 60 mg and 90 mg and 100 mg sitagliptin were tested alone or in combination, respectively. Of note, the medication intake was set one hour before the start of the oral glucose tolerance test and none of the medications led to hypoglycemia, although the study participants had fasted overnight. All medications were well tolerated.

The study revealed that the combination of low doses of DXM with sitagliptin further reduced blood glucose excursions during a glucose tolerance test in type 2 diabetics and increased early postprandial serum insulin concentrations compared to sitagliptin alone. In particular, all the combinations of DXM and sitagliptin numerically reduced maximum glucose concentrations compared to sitagliptin alone, with the combination of 60 mg DXM and sitagliptin showing a significant reduction. All three doses of DXM alone numerically reduced maximum glucose concentrations compared to placebo. Furthermore, the combinations exhibited higher values for maximum insulin concentrations compared to sitagliptin alone or placebo.

Regarding the course of serum insulin concentrations during the oral glucose tolerance test, this study revealed that the combinations of DXM 30 mg and 60 mg with sitagliptin respectively led to significantly higher serum insulin concentrations during the first 30 minutes of the test compared to sitagliptin alone. This effect could not be detected during the last two hours of the test, the late postprandial phase.

In sum, the clinical study presented in Marquard et al., 2016 is the first proof-of-concept study that shows that the combination of DXM together with sitagliptin has additive effects on reducing blood glucose concentrations and increasing serum insulin concentrations during an oral glucose tolerance test in type 2 diabetics.

4.2.2 Manuscript

See page 56 and following

Please note that the published version of this thesis does not contain the manuscript pages.

Reference:

Marquard, J., Stirban, A., Schliess, F., Sievers, F., Welters, A., Otter, S., Fischer, A., Wnendt, S., Meissner, T., Heise, T., and Lammert, E. (2016). Effects of dextromethorphan as add-on to sitagliptin on blood glucose and serum insulin concentrations in individuals with type 2 diabetes mellitus: a randomized, placebo-controlled, double-blinded, multiple crossover, single-dose clinical trial. Diabetes, obesity & metabolism 18, 100-103.
doi: 10.1111/dom.12576.

Weblink:

http://onlinelibrary.wiley.com/doi/10.1111/dom.12576/abstract;jsessionid=BDB95661615D4A261F94969EC57D1430.f02t04

4.2.3 Personal contribution and general information

Personal contribution of Silke Otter (SO) to the manuscript “Effects of dextromethorphan as add-on to sitagliptin on blood glucose and serum insulin concentrations in individuals with type 2 diabetes mellitus: a randomized, placebo-controlled, double-blinded, multiple crossover, single-dose clinical trial”:

Name of the journal: Diabetes, Obesity and Metabolism

Impact factor (2014): 6.36

Author position: sixth author

Tasks: preparation and correction of manuscript, figures, tables and supplement, contribution to study protocol writing

Author’s contribution adapted from the manuscript:

J. M. and E. L. designed the structure of manuscript and figures and wrote the manuscript with input from the other authors. J. M. generated figures and tables with input from E. L., A. S., A. W. and **S. O.** E. L. suggested to T. H. to perform a clinical trial on a combination of a low dose of DXM with a DPP-4 inhibitor, while T. H. suggested the use of sitagliptin. A.S., F. Sievers, F. Schliess, and T. H. guided the clinical trial whose protocol was written by A. S. with input from E. L., J. M., T. H., F. Schliess, T. M., A. W. and **S. O.** A. F. performed the statistical analyses. S. W. determined the insulin and glucagon concentrations. All authors were involved in drafting or revising the content of the paper and approved the final version of the manuscript.

Contribution in detail:

Silke Otter gave input to the design of the clinical trial protocol and contributed to writing and preparation of the manuscript, figures, tables and supplement.

In sum, Silke Otter contributed to the work of this manuscript with approximately 5-10%.

4.3 Exciting Times for Pancreatic Islets: Glutamate Signaling in Endocrine Cells (Otter and Lammert, 2016)

4.3.1 Summary

In this article, we review recent findings of the glutamate-mediated signaling in pancreatic islets including our findings published in Marquard et al., 2015 and Marquard et al., 2016. We discuss and combine the occasionally conflicting results and give rise to consensus models of intra- and extracellular glutamate-mediated signaling in the endocrine pancreas.

In detail, in this review we first describe general similarities between the endocrine cells of the pancreas and neurons but we also highlight the differences in glutamate signaling between these cell types.

We further discuss the different possibilities of intracellular glutamate formation in pancreatic beta cells, which includes the generation of glutamate in the cytosol via the malate-aspartate shuttle and the amination reaction catalyzed by glutamate dehydrogenase in the mitochondria to form glutamate from alpha-ketoglutarate and ammonia (Gheni et al., 2014; Maechler and Wollheim, 1999).

Additionally in Otter and Lammert, 2016 we suggest a consensus model of the role of intracellular glutamate in pancreatic beta cells and how intracellular glutamate can amplify glucose- and incretin-stimulated insulin secretion by combining the findings of different studies.

We then discuss the influence of extracellular glutamate signaling on pancreatic hormone secretion, and present a model of glutamate receptor signaling in the endocrine pancreas by embedding our findings from Marquard et al., 2015 into the context of the published literature.

At the end of Otter and Lammert, 2016 we point to the possibility that glutamate receptors in pancreatic islets, in particular NMDARs, may be used as drug targets for adjunct treatment of diabetes.

4.3.2 Manuscript

See page 67 and following

Please note that the published version of this thesis does not contain the manuscript pages.

Reference:

Otter, S., and Lammert, E. (2016). Exciting Times for Pancreatic Islets: Glutamate Signaling in Endocrine Cells. Trends in endocrinology and metabolism: TEM 27, 177-188.
doi: 10.1016/j.tem.2015.12.004.

Weblink:

http://www.sciencedirect.com/science/article/pii/S1043276015002489

4.3.3 Personal contribution and general information

Personal contribution of Silke Otter to the manuscript “Exciting Times for Pancreatic Islets: Glutamate Signaling in Endocrine Cells”:

Name of the journal: Trends in Endocrinology & Metabolism

Impact factor (2014): 9.392

Author position: first author

Tasks: literature research, manuscript writing and correction, figure preparation

Contribution in detail:

Silke Otter and Eckhard Lammert developed the structure and agreed on the content of this review. Silke Otter performed an extensive literature research, and Eckhard Lammert wrote the manuscript with assistance from Silke Otter. Correction and finalization of the manuscript was performed by both as well as the preparations of the figures. The artwork of the figures was done by Yousun Koh.

In sum, Silke Otter contributed to the work of this manuscript with 50 %.

4.4 Association of exercise-induced hyperinsulinaemic hypoglycaemia with *MCT1*-expressing insulinoma (Marquard et al., 2013)

4.4.1 Summary

In this article we reported a 16 year-old patient who suffered from symptoms of exercise-induced hyperinsulinism that were caused by an insulinoma which showed high expression of the *MCT1*-gene, encoding for the monocarboxylate transporter 1 (MCT1). This article showed for the first time that an insulinoma that was associated with exercise-induced hyperinsulinism exhibited *MCT1* expression and the presence of MCT1 protein.

In detail, an anaerobic exercise test was performed before surgical removal of the insulinoma and plasma glucose, serum insulin and plasma lactate concentrations were measured during and after anaerobic exercise. The measurements revealed that during the anaerobic exercise test a high rise in serum insulin concentrations was followed by a decline in plasma glucose concentrations resulting in a hypoglycemic event. Additionally, plasma lactate concentrations increased during the test. However, after surgical removal of the insulinoma a second anaerobic exercise test resulted in plasma glucose and serum insulin concentrations that were not altered during or after anaerobic exercise, although the plasma lactate concentrations were similar to those measured in the first test. The removed insulinoma tissue was subsequently analyzed using different methodological approaches. With the help of laser scanning microscopy, MCT1 protein could be detected in the insulinoma tissue. In contrast, MCT1 protein could not be detected in healthy islets of this patient with this method. On mRNA level an upregulation of *MCT1* expression compared to control human islets could be observed. Furthermore, western blot analysis confirmed the presence of MCT1 protein in the insulinoma of the patient and in three of four different insulinomas from other patients.

Our findings published in Marquard et al., 2013 support the hypothesis that expression of *MCT1* and the presence of MCT1 protein in human pancreatic islets is associated with exercise-induced hyperinsulinism. Furthermore, our data suggest that *MCT1* expression might be a common feature of the majority of insulinomas.

4.4.2 Manuscript

See page 82 and following

Please note that the published version of this thesis does not contain the manuscript pages.

Reference:

Marquard, J., Welters, A., Buschmann, T., Barthlen, W., Vogelgesang, S., Klee, D., Krausch, M., Raffel, A., Otter, S., Piemonti, L., Mayatepek, E., Otonkoski, T., Lammert, E., and Meissner, T. (2013). Association of exercise-induced hyperinsulinaemic hypoglycaemia with MCT1-expressing insulinoma. Diabetologia 56, 31-35.
doi: 10.1007/s00125-012-2750-7.

Weblink:

http://link.springer.com/article/10.1007%2Fs00125-012-2750-7

4.4.3 Personal contribution and general information

Personal contribution of Silke Otter (SO) to the manuscript “Association of exercise-induced hyperinsulinaemic hypoglycaemia with MCT1-expressing insulinoma”

Name of the journal: Diabetologia

Impact factor (2014): 6.671

Author position: ninth author

Tasks: acquisition of data, correction of manuscript

Author’s contribution adapted from the manuscript:

JM, AW, TM, TB, **SO**, LP, SV, MK, AR, DK and WB contributed to the acquisition of data. JM, AW, TM, EM, TO and EL made substantial contributions to the analysis and interpretation of data. EL, TM, TO, JM and AW contributed to the conception and design of the study. JM, AW, TM, TO and EL wrote the manuscript with critical input from EM, TB, **SO**, LP, DK, SV, MK, AR and WB. All authors approved the final version.

Contribution in detail:

Silke Otter collected and prepared lysates of human pancreatic islets from different donors for western blot analysis that were used to prepare Fig. 2 n,o,p.

In sum, Silke Otter contributed to the experimental work of this manuscript with approximately 5%.

5 Concluding remarks and outlook

Pancreatic islets, the endocrine mini-organs within the pancreas, play a pivotal role in the regulation of glucose homeostasis due to the secretion of different peptide hormones including insulin and glucagon. The amount and timing of hormone secretion must be adapted to the nutrient state of the body, in order to maintain blood glucose concentrations within a narrow range. Because of its blood glucose lowering effects, insulin secretion from pancreatic beta cells must be tightly controlled (Folias and Hebrok, 2014). On the one hand, this tight control is achieved by the ability of the pancreatic beta cells to act as nutrient sensor. However, it is known that many additional factors, such as neurotransmitters, in the framework of autocrine signaling, paracrine interactions or derived from the blood contribute to ascertain appropriate insulin secretion (Di Cairano et al., 2015). Dysfunction of pancreatic beta cells can cause inappropriate insulin secretion, which can lead to serious acute and long-term consequences (Folias and Hebrok, 2014). Beta cell dysfunction can be triggered by different factors such as lipotoxicity and glucotoxicity or can be caused by genetic defects (Welters and Lammert, 2014).

In Marquard et al., 2013 we reported a patient who suffered from recurrent hypoglycemia caused by exercise-induced hyperinsulinism. This condition was associated with an insulinoma expressing *MCT1* encoding a transporter for monocarboxylates, such as pyruvate and lactate. As *MCT1* expression and MCT1 protein is very low in normal pancreatic islets and beta cells (Otonkoski et al., 2007; Zhao et al., 2001), probably to avoid inappropriate insulin secretion by circulating metabolites, it seems to be likely that the detected protein levels of MCT1 triggered exercise-induced hyperinsulinemic hypoglycemia in this patient. In Marquard et al., 2013 we further investigated whether MCT1 protein was present in insulinomas of other patients. Indeed we could detect MCT1 protein in three of four different insulinomas of other patients. The detection of MCT1 protein in other insulinomas leads to the question whether *MCT1* expression and insulinoma formation occur independently or whether *MCT1* expression may contribute to insulinoma formation from pancreatic beta cells in humans. Interestingly, *MCT1* expression has been found to be increased in other tumors including breast and colon carcinomas compared to normal tissue (Pinheiro et al., 2012).

With regard to pancreatic hormone secretion, the role of intra- and extracellular glutamate has been in the focus of many research groups (Cabrera et al., 2008; Feldmann et al., 2011; Gheni et al., 2014; Maechler and Wollheim, 1999). Intracellular glutamate can increase glucose- as well as incretin-stimulated insulin secretion from pancreatic beta cells as discussed in Otter and Lammert, 2016. In these cells, glutamate is primarily formed via the malate-aspartate shuttle in the cytosol or by the mitochondrial enzyme glutamate dehydrogenase that catalyzes the addition of ammonia to alpha-ketoglutarate to form glutamate and vice versa (Gheni et al., 2014; Maechler and Wollheim, 1999). In this regard, it is of note that the molecular basis of the congenital hyperinsulinism involves activating mutations in *GLUD1* encoding for glutamate dehydrogenase. To date, mutations in nine genes have been identified to be associated with congenital hyperinsulinism. All these genes are involved in the regulation of insulin secretion from pancreatic beta cells (Rozenkova et al., 2015). Interestingly, only approximately 50% of all cases of congenital hyperinsulinism can be attributed to

mutations in these nine genes (Rahman et al., 2015). Given the important role of intracellular glutamate in insulin secretion, it may be worth investigating whether mutations in other genes involved in intracellular glutamate metabolism and signaling pathways may be associated with congenital hyperinsulinism.

Although it has been shown that extracellular glutamate can modulate pancreatic hormone secretion (Bertrand et al., 1992; Cabrera et al., 2008; Moriyama and Hayashi, 2003; Wu et al., 2012), the role of pancreatic NMDARs remained to be clarified, as the data, especially regarding the role of NMDARs in insulin secretion in humans, remained elusive and partly controversial (Inagaki et al., 1995; Konrad et al., 2000; Lechin et al., 2009; Molnar et al., 1995). Thus, in Marquard et al., 2015 we aimed to investigate the role of NMDARs in pancreatic islets regarding insulin secretion and islet cell survival. Our data indicate that pancreatic NMDARs are involved in the regulation of GSIS and blood glucose control. Inhibition of NMDARs in pancreatic islets resulted in increased GSIS, and pharmacological as well as genetic inhibition of NMDARs improved glucose tolerance *in vivo*. But what is the underlying molecular mechanism of how NMDARs are involved in the regulation of GSIS? Upon glucose stimulation and NMDAR inhibition, we could observe both a prolongation of the time beta cells spent in the depolarized state and a prolongation of the plateau fractions of Ca^{2+} oscillations in beta cells and isolated islets. The increasing effect of NMDAR inhibition on GSIS from pancreatic islets was dependent on the presence of functional K_{ATP} channels, was absent when VDCCs were pharmacologically blocked and was weaker in the absence of SK4 channels in pancreatic islets. Based on our findings from multiple experiments using pharmacological and genetical approaches we propose following model of how pancreatic NMDARs are involved in the regulation of GSIS: NMDARs that are localized on pancreatic beta cells get activated by extracellular glutamate and when the membrane is depolarized due to glucose stimulation. NMDAR activation then leads to the opening of K_{ATP} channels and SK4 channels. According to this model, the final net effect of NMDAR-mediated signaling is the repolarization of the glucose-stimulated and depolarized beta cell, which leads to the closure of Ca^{2+}-permissive VDCCs and thus limit GSIS (also see Supplementary Figure 8 in Marquard et al., 2015 and Figure 2 in Otter and Lammert, 2016). Together with the ligand- and voltage-gated character of NMDARs, this proposed model could explain the selectively increasing effect of NMDAR inhibition on GSIS in pancreatic islets, which leaves basal secretion largely unaffected, because at low glucose concentration beta cells are not electrically active (Ashcroft and Rorsman, 1990).

Although in Marquard et al., 2015 we could shed light on the molecular mechanism of how NMDARs can regulate insulin secretion, further work has to be done to fully elucidate this mechanism. For example the functional interaction of NMDARs with the K^+ channels needs to be further investigated. According to our model, NMDAR activation leads to the opening of K_{ATP} and SK4 channels. The opening of SK4 channels may be explained by Ca^{2+} influx through the activated NMDARs as SK4 channels belong to the family of Ca^{2+}-activated K^+ channels. Such an interaction between Ca^{2+}-activated K^+ channels and NMDARs has been shown in specific neurons (Isaacson and Murphy, 2001). Regarding the functional interaction of NMDARs and K_{ATP} channels, in rat subthalamic nucleus neurons, activation of NMDARs induces K_{ATP} channel opening mediated by second

messengers including nitric oxide (Shen and Johnson, 2010). Interestingly, in rat pancreatic beta cells, high concentrations of nitric oxide were shown to activate K_{ATP} channels (Sunouchi et al., 2008). However, it remains to be further investigated whether these signaling pathways for the opening of K^+ channels mediated by NMDAR activation also take place in pancreatic beta cells.

Furthermore, conducting two phase IIa clinical studies, we could show that single dose administration of the NMDAR antagonist DXM both at a high dose and at a low dose in combination with the DPP4 inhibitor sitagliptin improved blood glucose control and serum insulin concentrations during an oral glucose tolerance test in individuals with T2D (Marquard et al., 2015; Marquard et al., 2016). A typical feature of the insulin secretion pattern in type 2 diabetics is the impairment of the first-phase insulin secretion (Fehse et al., 2005). In this connection, it is of note that the combination of low dose DXM and Sitagliptin showed a higher increase of serum insulin concentrations during the first 30 min after starting the oral glucose tolerance test compared to placebo and sitagliptin alone (Marquard et al., 2016). The second clinical trial further points to the additive action of DXM and sitagliptin regarding their effects on blood glucose control and early postprandial serum insulin concentrations (Marquard et al., 2016).

Importantly, no hypoglycemic events were reported after administration of any dose of DXM, although the study participants had fasted overnight and drug administration took place one hour before starting the oral glucose tolerance test (Marquard et al., 2015; Marquard et al., 2016). This is in great contrast to sulfonylureas a class of commonly used antidiabetic drugs that harbor the risk of hypoglycemia (Tahrani et al., 2011).

The NMDAR antagonist DXM has been in clinical use as antitussive drug for several decades and has a good safety profile (Siu and Drachtman, 2007). Additionally, DXM is used for the treatment of diabetic neuropathic pain, nonketotic hyperglycinemia and pseudobulbar affect (Garnock-Jones, 2011; Hamosh et al., 1998; Nelson et al., 1997). During the clinical studies presented in Marquard et al., 2015 and Marquard et al., 2016 only mild to moderate adverse events were reported. However, in particular after administration of the high dose of DXM, central nervous system-related adverse events such as dizziness and headache could be observed. As our data indicate that the improving effect of NMDAR inhibition on insulin secretion and blood glucose control is mediated by pancreatic NMDARs, it would be interesting to test whether peripherally restricted NMDAR antagonists have less central nervous system-related adverse events without losing their antidiabetic potential (Marquard et al., 2015; Otter and Lammert, 2016).

Besides the direct effect on insulin secretion, we additionally investigated possible islet cell protective properties of NMDAR inhibition both *in vivo* and *in vitro*. Long-term treatment of a type 2 diabetic mouse model with DXM and treatment of isolated human islets from different donors with DXO and inflammatory cytokines indicate that NMDAR antagonists might have beneficial effects on islet cell function and survival under diabetogenic conditions. To date, there are no antidiabetic drugs available that can cure diabetes and show persistent beta cell protective effects in humans. Taking into account that in both, T1D and T2D a progressive decline of functional beta cells occurs, patients of both types of diabetes mellitus would benefit from an antidiabetic drug that has islet cell protective effects. As inflammatory cytokines are important pathogenic factors both in T2D and T1D (Donath et

al., 2003), it would be of great interest to investigate whether NMDAR antagonists have a delaying effect on the decline of beta cell function in type 1 as well as type 2 diabetics and thus on the progression of the disease.

Interestingly, in a preclinical study DXM treatment could reduce atherosclerosis in mice (Liu et al., 2009). Given the fact that individuals suffering from diabetes are prone to cardiovascular complications (ADA, 2013), it is worth investigating whether DXM besides its potential antidiabetic properties also harbors cardiovascular protective effects in humans (Otter and Lammert, 2016). An additional cardiovascular protective effect would be a significant advantage for an antidiabetic drug.

Our data suggest that NMDAR inhibition increases GSIS due to its effect on cytosolic Ca^{2+} oscillations. It has been proposed that Ca^{2+} signaling additionally is the mediator of the islet cell protective effects of NMDAR inhibition by stimulating the PI3K/Akt pathway via the Ca^{2+}-activated protein phosphatase calcineurin (Wollheim and Maechler, 2015). However, this has to be experimentally proven and more work has to be done to reveal the molecular mechanism of how NMDAR inhibition mediates cell protective effects in pancreatic islets.

Taken together, the regulation of insulin secretion from pancreatic beta cells is complex and comprises a plethora of signaling molecules in order to appropriately secrete insulin for the body's needs. Glutamate is one of these signaling molecules which modulates islet function and insulin secretion both intra- and extracellularly as discussed in Otter and Lammert, 2016. In Marquard et al., 2015 we have investigated the role of pancreatic NMDARs in insulin secretion and islet cell survival. Our data reveal that pancreatic NMDARs are involved in the regulation of insulin secretion and blood glucose control. Furthermore, our results indicate that pancreatic NMDARs act as negative regulators of insulin secretion and suggest that NMDAR antagonists have islet cell protective effects. In conclusion, our data reveal the possibility that pancreatic NMDARs represent potential new drug targets for the adjunct treatment of diabetes. However, further studies such as long-term clinical studies are required until NMDAR antagonists can be introduced to the clinic as safe antidiabetic drug.

6 References

ADA (2004). Gestational diabetes mellitus. Diabetes care 27 Suppl 1, S88-90.

ADA (2013). Diagnosis and classification of diabetes mellitus. Diabetes care 36 Suppl 1, S67-74.

Ardestani, A., Paroni, F., Azizi, Z., Kaur, S., Khobragade, V., Yuan, T., Frogne, T., Tao, W., Oberholzer, J., Pattou, F., Kerr Conte, J., and Maedler, K. (2014). MST1 is a key regulator of beta cell apoptosis and dysfunction in diabetes. Nature medicine 20, 385-397.

Arntfield, M.E., and van der Kooy, D. (2011). beta-Cell evolution: How the pancreas borrowed from the brain: The shared toolbox of genes expressed by neural and pancreatic endocrine cells may reflect their evolutionary relationship. BioEssays : news and reviews in molecular, cellular and developmental biology 33, 582-587.

Ashcroft, F.M., and Rorsman, P. (1990). ATP-sensitive K+ channels: a link between B-cell metabolism and insulin secretion. Biochemical Society transactions 18, 109-111.

Ashcroft, F.M., and Rorsman, P. (2012). Diabetes mellitus and the beta cell: the last ten years. Cell 148, 1160-1171.

Atkinson, M.A., Eisenbarth, G.S., and Michels, A.W. (2014). Type 1 diabetes. Lancet 383, 69-82.

Baggio, L.L., and Drucker, D.J. (2007). Biology of incretins: GLP-1 and GIP. Gastroenterology 132, 2131-2157.

Bedinger, D.H., and Adams, S.H. (2015). Metabolic, anabolic, and mitogenic insulin responses: A tissue-specific perspective for insulin receptor activators. Molecular and cellular endocrinology 415, 143-156.

Bertrand, G., Gross, R., Puech, R., Loubatieres-Mariani, M.M., and Bockaert, J. (1992). Evidence for a glutamate receptor of the AMPA subtype which mediates insulin release from rat perfused pancreas. British journal of pharmacology 106, 354-359.

Bertrand, G., Puech, R., Loubatieres-Mariani, M.M., and Bockaert, J. (1995). Glutamate stimulates insulin secretion and improves glucose tolerance in rats. The American journal of physiology 269, E551-556.

Beyerlein, A., Chmiel, R., Hummel, S., Winkler, C., Bonifacio, E., and Ziegler, A.G. (2014). Timing of gluten introduction and islet autoimmunity in young children: updated results from the BABYDIET study. Diabetes care 37, e194-195.

Braun, M., Ramracheya, R., Bengtsson, M., Clark, A., Walker, J.N., Johnson, P.R., and Rorsman, P. (2010). Gamma-aminobutyric acid (GABA) is an autocrine excitatory transmitter in human pancreatic beta-cells. Diabetes 59, 1694-1701.

Breton, M., Farret, A., Bruttomesso, D., Anderson, S., Magni, L., Patek, S., Dalla Man, C., Place, J., Demartini, S., Del Favero, S., Toffanin, C., Hughes-Karvetski, C., Dassau, E., Zisser, H., Doyle, F.J., 3rd, De Nicolao, G., Avogaro, A., Cobelli, C., Renard, E., and Kovatchev, B. (2012). Fully integrated artificial pancreas in type 1 diabetes: modular closed-loop glucose control maintains near normoglycemia. Diabetes 61, 2230-2237.

Brissova, M., Fowler, M.J., Nicholson, W.E., Chu, A., Hirshberg, B., Harlan, D.M., and Powers, A.C. (2005). Assessment of human pancreatic islet architecture and composition by laser scanning confocal microscopy. The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society 53, 1087-1097.

Bruning, J.C., Gautam, D., Burks, D.J., Gillette, J., Schubert, M., Orban, P.C., Klein, R., Krone, W., Muller-Wieland, D., and Kahn, C.R. (2000). Role of brain insulin receptor in control of body weight and reproduction. Science 289, 2122-2125.

Butler, A.E., Dhawan, S., Hoang, J., Cory, M., Zeng, K., Fritsch, H., Meier, J.J., Rizza, R.A., and Butler, P.C. (2016). beta-Cell Deficit in Obese Type 2 Diabetes, a Minor Role of beta-Cell Dedifferentiation and Degranulation. The Journal of clinical endocrinology and metabolism 101, 523-532.

Butler, A.E., Janson, J., Bonner-Weir, S., Ritzel, R., Rizza, R.A., and Butler, P.C. (2003). Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. Diabetes 52, 102-110.

Cabrera, O., Berman, D.M., Kenyon, N.S., Ricordi, C., Berggren, P.O., and Caicedo, A. (2006). The unique cytoarchitecture of human pancreatic islets has implications for islet cell function. Proceedings of the National Academy of Sciences of the United States of America 103, 2334-2339.

Cabrera, O., Jacques-Silva, M.C., Speier, S., Yang, S.N., Kohler, M., Fachado, A., Vieira, E., Zierath, J.R., Kibbey, R., Berman, D.M., Kenyon, N.S., Ricordi, C., Caicedo, A., and Berggren, P.O. (2008). Glutamate is a positive autocrine signal for glucagon release. Cell metabolism 7, 545-554.

Caicedo, A. (2013). Paracrine and autocrine interactions in the human islet: more than meets the eye. Seminars in cell & developmental biology 24, 11-21.

Cameron, F.J., and Wherrett, D.K. (2015). Care of diabetes in children and adolescents: controversies, changes, and consensus. Lancet 385, 2096-2106.

Cavaghan, M.K., Ehrmann, D.A., and Polonsky, K.S. (2000). Interactions between insulin resistance and insulin secretion in the development of glucose intolerance. The Journal of clinical investigation 106, 329-333.

Chandran, S., Yap, F., and Hussain, K. (2014). Molecular mechanisms of protein induced hyperinsulinaemic hypoglycaemia. World journal of diabetes 5, 666-677.

Cinti, F., Bouchi, R., Kim-Muller, J.Y., Ohmura, Y., Rodrigo Sandoval, P., Masini, M., Marselli, L., Suleiman, M., Ratner, L.E., Marchetti, P., and Accili, D. (2015). Evidence of beta-cell Dedifferentiation in Human Type 2 Diabetes. The Journal of clinical endocrinology and metabolism, jc20152860.

Cleveland, M.H., Sawyer, J.M., Afelik, S., Jensen, J., and Leach, S.D. (2012). Exocrine ontogenies: on the development of pancreatic acinar, ductal and centroacinar cells. Seminars in cell & developmental biology 23, 711-719.

Del Prato, S., and Marchetti, P. (2004). Beta- and alpha-cell dysfunction in type 2 diabetes. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme 36, 775-781.

Di Cairano, E.S., Davalli, A.M., Perego, L., Sala, S., Sacchi, V.F., La Rosa, S., Finzi, G., Placidi, C., Capella, C., Conti, P., Centonze, V.E., Casiraghi, F., Bertuzzi, F., Folli, F., and Perego, C. (2011). The glial glutamate transporter 1 (GLT1) is expressed by pancreatic beta-cells and prevents glutamate-induced beta-cell death. The Journal of biological chemistry 286, 14007-14018.

Di Cairano, E.S., Moretti, S., Marciani, P., Sacchi, V.F., Castagna, M., Davalli, A., Folli, F., and Perego, C. (2015). Neurotransmitters and Neuropeptides: New Players in the Control of Islet of Langerhans' Cell Mass and Function. Journal of cellular physiology. Published online August 31, 2015. doi:10.1002/jcp.25176.

Dicpinigaitis, P.V. (2015). Clinical perspective - cough: an unmet need. Current opinion in pharmacology 22, 24-28.

Dimitriadis, G., Mitrou, P., Lambadiari, V., Maratou, E., and Raptis, S.A. (2011). Insulin effects in muscle and adipose tissue. Diabetes research and clinical practice 93 Suppl 1, S52-59.

Donath, M.Y., Storling, J., Maedler, K., and Mandrup-Poulsen, T. (2003). Inflammatory mediators and islet beta-cell failure: a link between type 1 and type 2 diabetes. J Mol Med (Berl) 81, 455-470.

Drucker, D.J. (2006). The biology of incretin hormones. Cell metabolism 3, 153-165.

Drucker, D.J., and Nauck, M.A. (2006). The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. Lancet 368, 1696-1705.

Duca, L., Sippl, R., and Snell-Bergeon, J.K. (2013). Is the risk and nature of CVD the same in type 1 and type 2 diabetes? Current diabetes reports 13, 350-361.

Dufer, M., Gier, B., Wolpers, D., Krippeit-Drews, P., Ruth, P., and Drews, G. (2009). Enhanced glucose tolerance by SK4 channel inhibition in pancreatic beta-cells. Diabetes 58, 1835-1843.

Eberhard, D., Kragl, M., and Lammert, E. (2010). 'Giving and taking': endothelial and beta-cells in the islets of Langerhans. Trends in endocrinology and metabolism: TEM 21, 457-463.

Eberhard, D., Tosh, D., and Slack, J.M. (2008). Origin of pancreatic endocrine cells from biliary duct epithelium. Cellular and molecular life sciences : CMLS 65, 3467-3480.

Elrick, H., Stimmler, L., Hlad, C.J., Jr., and Arai, Y. (1964). Plasma Insulin Response to Oral and Intravenous Glucose Administration. The Journal of clinical endocrinology and metabolism 24, 1076-1082.

Farney, A.C., Sutherland, D.E., and Opara, E.C. (2016). Evolution of Islet Transplantation for the Last 30 Years. Pancreas 45, 8-20.

Fava, E., Dehghany, J., Ouwendijk, J., Muller, A., Niederlein, A., Verkade, P., Meyer-Hermann, M., and Solimena, M. (2012). Novel standards in the measurement of rat insulin granules combining electron microscopy, high-content image analysis and in silico modelling. Diabetologia 55, 1013-1023.

Fehse, F., Trautmann, M., Holst, J.J., Halseth, A.E., Nanayakkara, N., Nielsen, L.L., Fineman, M.S., Kim, D.D., and Nauck, M.A. (2005). Exenatide augments first- and second-phase insulin secretion in response to intravenous glucose in subjects with type 2 diabetes. The Journal of clinical endocrinology and metabolism 90, 5991-5997.

Feldmann, N., del Rio, R.M., Gjinovci, A., Tamarit-Rodriguez, J., Wollheim, C.B., and Wiederkehr, A. (2011). Reduction of plasma membrane glutamate transport potentiates insulin but not glucagon secretion in pancreatic islet cells. Molecular and cellular endocrinology 338, 46-57.

Ferrannini, E. (2010). The stunned beta cell: a brief history. Cell metabolism 11, 349-352.

Folias, A.E., and Hebrok, M. (2014). Pancreas Overview. In Metabolism of Human Diseases. E. Lammert, and M. Zeeb, eds. (Springer-Verlag Wien), pp. 157-162.

Gaisano, H.Y. (2014). Here come the newcomer granules, better late than never. Trends in endocrinology and metabolism: TEM 25, 381-388.

Garnock-Jones, K.P. (2011). Dextromethorphan/quinidine: in pseudobulbar affect. CNS drugs 25, 435-445.

Garrino, M.G., and Henquin, J.C. (1987). Adamantane derivatives: a new class of insulin secretagogues. British journal of pharmacology 90, 583-591.

Gheni, G., Ogura, M., Iwasaki, M., Yokoi, N., Minami, K., Nakayama, Y., Harada, K., Hastoy, B., Wu, X., Takahashi, H., Kimura, K., Matsubara, T., Hoshikawa, R., Hatano, N., Sugawara, K., Shibasaki,

T., Inagaki, N., Bamba, T., Mizoguchi, A., Fukusaki, E., Rorsman, P., and Seino, S. (2014). Glutamate acts as a key signal linking glucose metabolism to incretin/cAMP action to amplify insulin secretion. Cell reports 9, 661-673.

Goldstein, B.J. (2002). Insulin resistance as the core defect in type 2 diabetes mellitus. The American journal of cardiology 90, 3G-10G.

Gonoi, T., Mizuno, N., Inagaki, N., Kuromi, H., Seino, Y., Miyazaki, J., and Seino, S. (1994). Functional neuronal ionotropic glutamate receptors are expressed in the non-neuronal cell line MIN6. The Journal of biological chemistry 269, 16989-16992.

Grarup, N., Sandholt, C.H., Hansen, T., and Pedersen, O. (2014). Genetic susceptibility to type 2 diabetes and obesity: from genome-wide association studies to rare variants and beyond. Diabetologia 57, 1528-1541.

Guemes, M., and Hussain, K. (2015). Hyperinsulinemic Hypoglycemia. Pediatric clinics of North America 62, 1017-1036.

Guilherme, A., Virbasius, J.V., Puri, V., and Czech, M.P. (2008). Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. Nature reviews. Molecular cell biology 9, 367-377.

Halestrap, A.P., and Price, N.T. (1999). The proton-linked monocarboxylate transporter (MCT) family: structure, function and regulation. The Biochemical journal 343 Pt 2, 281-299.

Hamosh, A., Maher, J.F., Bellus, G.A., Rasmussen, S.A., and Johnston, M.V. (1998). Long-term use of high-dose benzoate and dextromethorphan for the treatment of nonketotic hyperglycinemia. The Journal of pediatrics 132, 709-713.

Harding, J.L., Shaw, J.E., Peeters, A., Cartensen, B., and Magliano, D.J. (2015). Cancer risk among people with type 1 and type 2 diabetes: disentangling true associations, detection bias, and reverse causation. Diabetes care 38, 264-270.

Henquin, J.C. (2009). Regulation of insulin secretion: a matter of phase control and amplitude modulation. Diabetologia 52, 739-751.

Hertz, L. (2006). Glutamate, a neurotransmitter--and so much more. A synopsis of Wierzba III. Neurochemistry international 48, 416-425.

Holst, J.J. (2007). The physiology of glucagon-like peptide 1. Physiological reviews 87, 1409-1439.

Holt, R.I.G., and Hanley, N.A. (2007). Overview of diabetes. In Essential Endocrinology and Diabetes (Blackwell Publishing), pp. 183-196.

Inagaki, N., Kuromi, H., Gonoi, T., Okamoto, Y., Ishida, H., Seino, Y., Kaneko, T., Iwanaga, T., and Seino, S. (1995). Expression and role of ionotropic glutamate receptors in pancreatic islet cells. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 9, 686-691.

Insel, R.A., Dunne, J.L., and Ziegler, A.G. (2015). General population screening for type 1 diabetes: has its time come? Current opinion in endocrinology, diabetes, and obesity 22, 270-276.

Isaacson, J.S., and Murphy, G.J. (2001). Glutamate-mediated extrasynaptic inhibition: direct coupling of NMDA receptors to Ca(2+)-activated K+ channels. Neuron 31, 1027-1034.

Jansson, L., and Hellerstrom, C. (1986). Glucose-induced changes in pancreatic islet blood flow mediated by central nervous system. The American journal of physiology 251, E644-647.

Jiang, G., and Zhang, B.B. (2003). Glucagon and regulation of glucose metabolism. American journal of physiology. Endocrinology and metabolism 284, E671-678.

Kahn, S.E., Cooper, M.E., and Del Prato, S. (2014). Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present, and future. Lancet 383, 1068-1083.

Kahn, S.E., Hull, R.L., and Utzschneider, K.M. (2006). Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 444, 840-846.

Kailey, B., van de Bunt, M., Cheley, S., Johnson, P.R., MacDonald, P.E., Gloyn, A.L., Rorsman, P., and Braun, M. (2012). SSTR2 is the functionally dominant somatostatin receptor in human pancreatic beta- and alpha-cells. American journal of physiology. Endocrinology and metabolism 303, E1107-1116.

Kalia, L.V., Kalia, S.K., and Salter, M.W. (2008). NMDA receptors in clinical neurology: excitatory times ahead. The Lancet. Neurology 7, 742-755.

Kapoor, R.R., Flanagan, S.E., Arya, V.B., Shield, J.P., Ellard, S., and Hussain, K. (2013). Clinical and molecular characterisation of 300 patients with congenital hyperinsulinism. European journal of endocrinology / European Federation of Endocrine Societies 168, 557-564.

Karlsson, H.K., and Zierath, J.R. (2007). Insulin signaling and glucose transport in insulin resistant human skeletal muscle. Cell biochemistry and biophysics 48, 103-113.

Knip, M., Akerblom, H.K., Becker, D., Dosch, H.M., Dupre, J., Fraser, W., Howard, N., Ilonen, J., Krischer, J.P., Kordonouri, O., Lawson, M.L., Palmer, J.P., Savilahti, E., Vaarala, O., and Virtanen, S.M. (2014). Hydrolyzed infant formula and early beta-cell autoimmunity: a randomized clinical trial. Jama 311, 2279-2287.

Koh, D.S., Cho, J.H., and Chen, L. (2012). Paracrine interactions within islets of Langerhans. Journal of molecular neuroscience : MN 48, 429-440.

Konrad, D., Sobetzko, D., Schmitt, B., and Schoenle, E.J. (2000). Insulin-dependent diabetes mellitus induced by the antitussive agent dextromethorphan. Diabetologia 43, 261-262.

Lammert, E., Gu, G., McLaughlin, M., Brown, D., Brekken, R., Murtaugh, L.C., Gerber, H.P., Ferrara, N., and Melton, D.A. (2003). Role of VEGF-A in vascularization of pancreatic islets. Current biology : CB 13, 1070-1074.

Lechin, F., van der Dijs, B., Pardey-Maldonado, B., Rivera, J.E., Lechin, M.E., and Baez, S. (2009). Amantadine reduces glucagon and enhances insulin secretion throughout the oral glucose tolerance test: central plus peripheral nervous system mechanisms. Diabetes, metabolic syndrome and obesity : targets and therapy 2, 203-213.

Lenzen, S. (2014). A fresh view of glycolysis and glucokinase regulation: history and current status. The Journal of biological chemistry 289, 12189-12194.

Leon, B.M., and Maddox, T.M. (2015). Diabetes and cardiovascular disease: Epidemiology, biological mechanisms, treatment recommendations and future research. World journal of diabetes 6, 1246-1258.

Li, Y.V. (2014). Zinc and insulin in pancreatic beta-cells. Endocrine 45, 178-189.

Liu, J.J., Lee, T., and DeFronzo, R.A. (2012). Why Do SGLT2 inhibitors inhibit only 30-50% of renal glucose reabsorption in humans? Diabetes 61, 2199-2204.

Liu, S.L., Li, Y.H., Shi, G.Y., Tang, S.H., Jiang, S.J., Huang, C.W., Liu, P.Y., Hong, J.S., and Wu, H.L. (2009). Dextromethorphan reduces oxidative stress and inhibits atherosclerosis and neointima formation in mice. Cardiovascular research 82, 161-169.

Low, J.T., Shukla, A., and Thorn, P. (2010). Pancreatic acinar cell: new insights into the control of secretion. The international journal of biochemistry & cell biology 42, 1586-1589.

Maechler, P. (2013). Mitochondrial function and insulin secretion. Molecular and cellular endocrinology 379, 12-18.

Maechler, P., and Wollheim, C.B. (1999). Mitochondrial glutamate acts as a messenger in glucose-induced insulin exocytosis. Nature 402, 685-689.

Marquard, J., Otter, S., Welters, A., Stirban, A., Fischer, A., Eglinger, J., Herebian, D., Kletke, O., Klemen, M.S., Stozer, A., Wnendt, S., Piemonti, L., Kohler, M., Ferrer, J., Thorens, B., Schliess, F., Rupnik, M.S., Heise, T., Berggren, P.O., Klocker, N., Meissner, T., Mayatepek, E., Eberhard, D., Kragl, M., and Lammert, E. (2015). Characterization of pancreatic NMDA receptors as possible drug targets for diabetes treatment. Nature medicine 21, 363-372.

Marquard, J., Stirban, A., Schliess, F., Sievers, F., Welters, A., Otter, S., Fischer, A., Wnendt, S., Meissner, T., Heise, T., and Lammert, E. (2016). Effects of dextromethorphan as add-on to sitagliptin on blood glucose and serum insulin concentrations in individuals with type 2 diabetes mellitus: a randomized, placebo-controlled, double-blinded, multiple crossover, single-dose clinical trial. Diabetes, obesity & metabolism 18, 100-103.

Marquard, J., Welters, A., Buschmann, T., Barthlen, W., Vogelgesang, S., Klee, D., Krausch, M., Raffel, A., Otter, S., Piemonti, L., Mayatepek, E., Otonkoski, T., Lammert, E., and Meissner, T. (2013). Association of exercise-induced hyperinsulinaemic hypoglycaemia with MCT1-expressing insulinoma. Diabetologia 56, 31-35.

McCall, M., and Shapiro, A.M. (2014). Islet cell transplantation. Seminars in pediatric surgery 23, 83-90.

McCulloch, L.J., van de Bunt, M., Braun, M., Frayn, K.N., Clark, A., and Gloyn, A.L. (2011). GLUT2 (SLC2A2) is not the principal glucose transporter in human pancreatic beta cells: implications for understanding genetic association signals at this locus. Molecular genetics and metabolism 104, 648-653.

Mergenthaler, P., Lindauer, U., Dienel, G.A., and Meisel, A. (2013). Sugar for the brain: the role of glucose in physiological and pathological brain function. Trends in neurosciences 36, 587-597.

Molina, J., Rodriguez-Diaz, R., Fachado, A., Jacques-Silva, M.C., Berggren, P.O., and Caicedo, A. (2014). Control of insulin secretion by cholinergic signaling in the human pancreatic islet. Diabetes 63, 2714-2726.

Molnar, E., Varadi, A., McIlhinney, R.A., and Ashcroft, S.J. (1995). Identification of functional ionotropic glutamate receptor proteins in pancreatic beta-cells and in islets of Langerhans. FEBS letters 371, 253-257.

Moriyama, Y., and Hayashi, M. (2003). Glutamate-mediated signaling in the islets of Langerhans: a thread entangled. Trends in pharmacological sciences 24, 511-517.

Nanto-Salonen, K., Kupila, A., Simell, S., Siljander, H., Salonsaari, T., Hekkala, A., Korhonen, S., Erkkola, R., Sipila, J.I., Haavisto, L., Siltala, M., Tuominen, J., Hakalax, J., Hyoty, H., Ilonen, J., Veijola, R., Simell, T., Knip, M., and Simell, O. (2008). Nasal insulin to prevent type 1 diabetes in children with HLA genotypes and autoantibodies conferring increased risk of disease: a double-blind, randomised controlled trial. Lancet 372, 1746-1755.

Nathan, D.M. (2015). Diabetes: Advances in Diagnosis and Treatment. Jama 314, 1052-1062.

Nathan, D.M., Buse, J.B., Davidson, M.B., Ferrannini, E., Holman, R.R., Sherwin, R., and Zinman, B. (2009). Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes care 32, 193-203.

Nelson, K.A., Park, K.M., Robinovitz, E., Tsigos, C., and Max, M.B. (1997). High-dose oral dextromethorphan versus placebo in painful diabetic neuropathy and postherpetic neuralgia. Neurology 48, 1212-1218.

Noble, J.A., Valdes, A.M., Varney, M.D., Carlson, J.A., Moonsamy, P., Fear, A.L., Lane, J.A., Lavant, E., Rappner, R., Louey, A., Concannon, P., Mychaleckyj, J.C., and Erlich, H.A. (2010). HLA class I and genetic susceptibility to type 1 diabetes: results from the Type 1 Diabetes Genetics Consortium. Diabetes 59, 2972-2979.

Nolan, C.J., Damm, P., and Prentki, M. (2011). Type 2 diabetes across generations: from pathophysiology to prevention and management. Lancet 378, 169-181.

Otonkoski, T., Jiao, H., Kaminen-Ahola, N., Tapia-Paez, I., Ullah, M.S., Parton, L.E., Schuit, F., Quintens, R., Sipila, I., Mayatepek, E., Meissner, T., Halestrap, A.P., Rutter, G.A., and Kere, J. (2007). Physical exercise-induced hypoglycemia caused by failed silencing of monocarboxylate transporter 1 in pancreatic beta cells. American journal of human genetics 81, 467-474.

Otonkoski, T., Kaminen, N., Ustinov, J., Lapatto, R., Meissner, T., Mayatepek, E., Kere, J., and Sipila, I. (2003). Physical exercise-induced hyperinsulinemic hypoglycemia is an autosomal-dominant trait characterized by abnormal pyruvate-induced insulin release. Diabetes 52, 199-204.

Otter, S., and Lammert, E. (2016). Exciting Times for Pancreatic Islets: Glutamate Signaling in Endocrine Cells. Trends in endocrinology and metabolism: TEM 27, 177-188.

Paoletti, P., Bellone, C., and Zhou, Q. (2013). NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease. Nature reviews. Neuroscience 14, 383-400.

Perry, R.J., Samuel, V.T., Petersen, K.F., and Shulman, G.I. (2014). The role of hepatic lipids in hepatic insulin resistance and type 2 diabetes. Nature 510, 84-91.

Pinheiro, C., Longatto-Filho, A., Azevedo-Silva, J., Casal, M., Schmitt, F.C., and Baltazar, F. (2012). Role of monocarboxylate transporters in human cancers: state of the art. Journal of bioenergetics and biomembranes 44, 127-139.

Plaitakis, A., Zaganas, I., and Spanaki, C. (2013). Deregulation of glutamate dehydrogenase in human neurologic disorders. Journal of neuroscience research 91, 1007-1017.

Plum, L., Schubert, M., and Bruning, J.C. (2005). The role of insulin receptor signaling in the brain. Trends in endocrinology and metabolism: TEM 16, 59-65.

Prentki, M., Matschinsky, F.M., and Madiraju, S.R. (2013). Metabolic signaling in fuel-induced insulin secretion. Cell metabolism 18, 162-185.

Pullen, T.J., Sylow, L., Sun, G., Halestrap, A.P., Richter, E.A., and Rutter, G.A. (2012). Overexpression of monocarboxylate transporter-1 (SLC16A1) in mouse pancreatic beta-cells leads to relative hyperinsulinism during exercise. Diabetes 61, 1719-1725.

Rahman, S.A., Nessa, A., and Hussain, K. (2015). Molecular mechanisms of congenital hyperinsulinism. Journal of molecular endocrinology 54, R119-129.

Rhodes, C.J. (2005). Type 2 diabetes-a matter of beta-cell life and death? Science 307, 380-384.

Rhodes, C.J., White, M.F., Leahy, J.L., and Kahn, S.E. (2013). Direct autocrine action of insulin on beta-cells: does it make physiological sense? Diabetes 62, 2157-2163.

Rodriguez-Diaz, R., Abdulreda, M.H., Formoso, A.L., Gans, I., Ricordi, C., Berggren, P.O., and Caicedo, A. (2011a). Innervation patterns of autonomic axons in the human endocrine pancreas. Cell metabolism 14, 45-54.

Rodriguez-Diaz, R., Dando, R., Jacques-Silva, M.C., Fachado, A., Molina, J., Abdulreda, M.H., Ricordi, C., Roper, S.D., Berggren, P.O., and Caicedo, A. (2011b). Alpha cells secrete acetylcholine as a non-neuronal paracrine signal priming beta cell function in humans. Nature medicine 17, 888-892.

Rorsman, P., and Braun, M. (2013). Regulation of insulin secretion in human pancreatic islets. Annual review of physiology 75, 155-179.

Rorsman, P., and Renstrom, E. (2003). Insulin granule dynamics in pancreatic beta cells. Diabetologia 46, 1029-1045.

Rozenkova, K., Guemes, M., Shah, P., and Hussain, K. (2015). The Diagnosis and Management of Hyperinsulinaemic Hypoglycaemia. Journal of clinical research in pediatric endocrinology 7, 86-97.

Rutter, G.A., Pullen, T.J., Hodson, D.J., and Martinez-Sanchez, A. (2015). Pancreatic beta-cell identity, glucose sensing and the control of insulin secretion. The Biochemical journal 466, 203-218.

Ryan, E.A., Paty, B.W., Senior, P.A., Bigam, D., Alfadhli, E., Kneteman, N.M., Lakey, J.R., and Shapiro, A.M. (2005). Five-year follow-up after clinical islet transplantation. Diabetes 54, 2060-2069.

Saltiel, A.R., and Kahn, C.R. (2001). Insulin signalling and the regulation of glucose and lipid metabolism. Nature 414, 799-806.

Satin, L.S., Butler, P.C., Ha, J., and Sherman, A.S. (2015). Pulsatile insulin secretion, impaired glucose tolerance and type 2 diabetes. Molecular aspects of medicine 42, 61-77.

Shen, K.Z., and Johnson, S.W. (2010). Ca2+ influx through NMDA-gated channels activates ATP-sensitive K+ currents through a nitric oxide-cGMP pathway in subthalamic neurons. The Journal of neuroscience : the official journal of the Society for Neuroscience 30, 1882-1893.

Siddle, K. (2011). Signalling by insulin and IGF receptors: supporting acts and new players. Journal of molecular endocrinology 47, R1-10.

Siu, A., and Drachtman, R. (2007). Dextromethorphan: a review of N-methyl-d-aspartate receptor antagonist in the management of pain. CNS drug reviews 13, 96-106.

Sunouchi, T., Suzuki, K., Nakayama, K., and Ishikawa, T. (2008). Dual effect of nitric oxide on ATP-sensitive K+ channels in rat pancreatic beta cells. Pflugers Archiv : European journal of physiology 456, 573-579.

Tahrani, A.A., Bailey, C.J., Del Prato, S., and Barnett, A.H. (2011). Management of type 2 diabetes: new and future developments in treatment. Lancet 378, 182-197.

Tahrani, A.A., Piya, M.K., Kennedy, A., and Barnett, A.H. (2010). Glycaemic control in type 2 diabetes: targets and new therapies. Pharmacology & therapeutics 125, 328-361.

Talchai, C., Xuan, S., Lin, H.V., Sussel, L., and Accili, D. (2012). Pancreatic beta cell dedifferentiation as a mechanism of diabetic beta cell failure. Cell 150, 1223-1234.

Taniguchi, C.M., Emanuelli, B., and Kahn, C.R. (2006). Critical nodes in signalling pathways: insights into insulin action. Nature reviews. Molecular cell biology 7, 85-96.

Tengholm, A., and Gylfe, E. (2009). Oscillatory control of insulin secretion. Molecular and cellular endocrinology 297, 58-72.

Thanabalasingham, G., and Owen, K.R. (2011). Diagnosis and management of maturity onset diabetes of the young (MODY). BMJ 343, d6044.

Traynelis, S.F., Wollmuth, L.P., McBain, C.J., Menniti, F.S., Vance, K.M., Ogden, K.K., Hansen, K.B., Yuan, H., Myers, S.J., and Dingledine, R. (2010). Glutamate receptor ion channels: structure, regulation, and function. Pharmacological reviews 62, 405-496.

Tuomi, T., Santoro, N., Caprio, S., Cai, M., Weng, J., and Groop, L. (2014). The many faces of diabetes: a disease with increasing heterogeneity. Lancet 383, 1084-1094.

van Arensbergen, J., Garcia-Hurtado, J., Moran, I., Maestro, M.A., Xu, X., Van de Casteele, M., Skoudy, A.L., Palassini, M., Heimberg, H., and Ferrer, J. (2010). Derepression of Polycomb targets during pancreatic organogenesis allows insulin-producing beta-cells to adopt a neural gene activity program. Genome research 20, 722-732.

van Belle, T.L., Coppieters, K.T., and von Herrath, M.G. (2011). Type 1 diabetes: etiology, immunology, and therapeutic strategies. Physiological reviews 91, 79-118.

Vetere, A., Choudhary, A., Burns, S.M., and Wagner, B.K. (2014). Targeting the pancreatic beta-cell to treat diabetes. Nature reviews. Drug discovery 13, 278-289.

Vetterli, L., Carobbio, S., Pournourmohammadi, S., Martin-Del-Rio, R., Skytt, D.M., Waagepetersen, H.S., Tamarit-Rodriguez, J., and Maechler, P. (2012). Delineation of glutamate pathways and secretory responses in pancreatic islets with beta-cell-specific abrogation of the glutamate dehydrogenase. Molecular biology of the cell 23, 3851-3862.

Wang, X., Strizich, G., Hu, Y., Wang, T., Kaplan, R.C., and Qi, Q. (2016). Genetic markers of type 2 diabetes: Progress in genome-wide association studies and clinical application for risk prediction. Journal of diabetes 8, 24-35.

Weaver, C.D., Yao, T.L., Powers, A.C., and Verdoorn, T.A. (1996). Differential expression of glutamate receptor subtypes in rat pancreatic islets. The Journal of biological chemistry 271, 12977-12984.

Welters, A., and Lammert, E. (2014). Diabetes Mellitus. In Metabolism of Human Diseases. E. Lammert, and M. Zeeb, eds. (Springer-Verlag Wien), pp. 163-169.

WHO (2008). The global burden of disease: 2004 update. (Geneva: World Health Organization).

Wollheim, C.B., and Maechler, P. (2015). Beta cell glutamate receptor antagonists: novel oral antidiabetic drugs? Nature medicine 21, 310-311.

Wu, Z.Y., Zhu, L.J., Zou, N., Bombek, L.K., Shao, C.Y., Wang, N., Wang, X.X., Liang, L., Xia, J., Rupnik, M., and Shen, Y. (2012). AMPA receptors regulate exocytosis and insulin release in pancreatic beta cells. Traffic 13, 1124-1139.

Yang, S.N., Shi, Y., Yang, G., Li, Y., Yu, J., and Berggren, P.O. (2014). Ionic mechanisms in pancreatic beta cell signaling. Cellular and molecular life sciences : CMLS 71, 4149-4177.

Zhao, C., Wilson, M.C., Schuit, F., Halestrap, A.P., and Rutter, G.A. (2001). Expression and distribution of lactate/monocarboxylate transporter isoforms in pancreatic islets and the exocrine pancreas. Diabetes 50, 361-366.

List of abbreviations

SI units and their symbols were used throughout this thesis.

ABCC8	encodes the SUR1 subunit of the K_{ATP} channel
ADA	American Diabetes Association
ADP	adenosine diphosphate
Akt	also known as protein kinase B
AMPAR	alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
ATP	adenosine triphosphate
Ca^{2+}	calcium
cAMP	cyclic adenosine monophosphate
DPP4	dipeptidyl peptidase-4
DXM	dextromethorphan
DXO	dextrorphan
ER	endoplasmic reticulum
Epac2	exchange protein directly activated by cAMP 2, also known as cAMP-guanine nucleotide exchange factor 2
GABA	gamma-aminobutyric acid
GCK	encodes the enzyme glucokinase
GIP	glucose-dependent insulinotropic polypeptide
GLP-1	glucagon-like peptide 1
GLUD1	encodes the enzyme glutamate dehydrogenase
GLUT1-4	glucose transporter 1-4
GSIS	glucose-stimulated insulin secretion
GTP	guanosine triphosphate
HbA1c	glycated hemoglobin
HLA	human leukocyte antigen
K^+	potassium
K_{ATP}	ATP-sensitive K^+ channel
KCNJ11	encodes the Kir6.2 subunit of the K_{ATP} channel
Kir6.2	inwardly-rectifying K^+ channel 6.2
Mg^{2+}	magnesium
MAPK	mitogen-activated protein kinase
MCP-1	monocyte chemoattractant protein-1
MCT1	monocarboxylate transporter 1
MODY	maturity-onset diabetes of the young
mRNA	messenger ribonucleic acid
Na^+	sodium
NAD(H)	nicotinamide adenine dinucleotide

NADP(H)	nicotinamide adenine dinucleotide phosphate
NMDA	N-methyl-D-aspartate
NMDAR	N-methyl-D-aspartate receptor
NEFA	non-esterified fatty acid
PI3K	phosphatidylinositol 3-kinase
PKA	protein kinase A
PP	pancreatic polypeptide
RRP	readily releasable pool
SGLT2	Na^+-glucose co-transporter 2
SK4	small conductance Ca^{2+}-activated K^+ channel 4
SLC16A1/MCT1	encodes the transporter MCT1
SNARE	soluble N-ethylmaleimide-sensitive factor attachment protein receptor
SUR1	sulfonylurea receptor 1
T1D	type 1 diabetes mellitus
T2D	type 2 diabetes mellitus
TCF7L2	encodes transcription factor 7-like 2
TNF-alpha	tumor necrosis factor alpha
VDCC	voltage-dependent Ca^{2+} channel
VEGF-A	vascular endothelial growth factor A
WHO	World Health Organization
Zn^{2+}	zinc

Acknowledgements

First of all, I would like to thank my supervisor Prof. Dr. Eckhard Lammert
for giving me the opportunity to write my thesis in his lab, for his guidance and supervision, for giving me good advise and for providing a productive work environment.

I would like to thank Prof. Philipp A. Lang
for being my second referee and for giving me good advise.

I would like to thank all the present and former members of the lab in particular

Dr. Alena Welters
for all your advise and help,
for all the productive conversations and motivating words.

Dr. Martin Kragl and Dr. Daniel Eberhard
for all your help and for many great conversations, both scientific and otherwise.

Dr. Jan Eglinger
for all that I have learned from you and for always answering my questions.

Jennifer Axnick
for being the best “bench-mate” and for always having an open ear.

Dr. Jan Marquard
for training and handing a great project over to me.

Barbara Bartosinska and Silke Jakob
for all your help and support, both technical and otherwise.

as well as Sofia Urner, Astrid Wies, Jessica Mrugala, Okka Scholz, Tobias Buschmann, Dr. Lara Planas-Paz, Dr. Ruchi Jain, Dr. Deepak Jain, Carina Klüppel, Anna Branopolski, Esther Hedderich, Dr. Martin Zeeb, Haiko Karsjens, Fatma Demir, Isabelle Hermanski, Dr. Bengt-F. Belgardt, Paul Schauerte, Linda Lorenz for your support and the great time in the lab.

Furthermore, I would like to thank PD Dr. Thomas Meissner, Prof. Dr. Ertan Mayatepek, Anja Leinweber and all our collaborators, especially Dr. Martin Köhler, Dr. Andraz Stozer, Dr. Masa Skelin Klemen, Prof. Dr. Marjan Slak Rupnik, Prof. Dr. Per-Olof Berggren, Prof. Dr. Nikolaj Klöcker, Dr. Olaf Kletke and the members of Profil Institute Neuss, especially Annelie Fischer for all their help.

I would like to thank Susanne Mansfeld and all other members of the graduate school “vivid”
for helpful discussions and the fantastic time that we have spent together.

I would like to thank my family and friends in particular

André Wölk
for always supporting me, for giving me all your love.

my parents Elisabeth Otter and Bernhard Otter
for supporting me throughout my life, for always believing in me.

Kathrin Pollmann, Ann-Christine Güth, Verena Schmidt, Nicole Görldt, Melissa Kühn, Julien Ostermann, you are the best.

Declaration

Eidesstattliche Erklärung

Ich versichere an Eides Statt, dass die Dissertation von mir selbstständig und ohne unzulässige fremde Hilfe unter Beachtung der „Grundsätze zur Sicherung guter wissenschaftlicher Praxis an der Heinrich-Heine-Universität Düsseldorf“ erstellt worden ist. Ich habe keine anderen als die angegebenen Quellen und Hilfsmittel verwendet und habe alle Stellen, in denen ich Bezug auf die Arbeit Anderer nehme, als solche kenntlich gemacht.

Die vorliegende Dissertation wurde weder in dieser noch in ähnlicher Form von mir bei einer anderen Institution eingereicht.

Vorherige Versuche zur Promotion wurden von mir nicht unternommen.

Gezeichnet

Silke Otter

Düsseldorf im Februar 2016

Declaration

I declare on oath that I have prepared the thesis independently and without forbidden outside help and according to the “Good Scientific Practice of the Heinrich-Heine-University Düsseldorf”. I did not use other sources and aid than those indicated and I marked all positions, where I refer to the work of others.

I did not submit this thesis in this form or modified to any other institution.

I did not make any previous attempts to do a PhD.

Signed

Silke Otter

Düsseldorf in February 2016