Pathomechanisms of insulin secretion disorders: Role of pancreatic NMDA receptors in diabetes mellitus and aberrant expression of *MCT1* in hyperinsulinaemic hypoglycaemia

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Dr. med. Alena Welters
aus Münster (Westfalen)

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aus dem Institut für Stoffwechselphysiologie
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Referent: Prof. Dr. Eckhard Lammert

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This thesis consists of four independent publications that have been published between 2013 and 2015:

- **Characterization of pancreatic NMDA receptors as possible drug targets for diabetes treatment *(Nat Med 2015 Apr;21(4):363-72)***
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  *Authors contributed equally to this work

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  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
  *Authors contributed equally to this work

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  A. Welters, E. Lammert

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Abstracts

Abstract

Carbohydrates are one of the human's three main energy sources, and glucose is an essential metabolic fuel for the brain. Both, low blood glucose concentrations (hypoglycaemia) as well as elevated blood glucose concentrations (hyperglycaemia) are associated with acute life-threatening events (coma) and may induce long-term complications such as permanent brain injury (hypoglycaemia) or cardiovascular disease (hyperglycaemia) when existing over time. It is therefore important to maintain blood glucose concentration within a narrow range (glucose homeostasis). The control of glucose homeostasis is primarily achieved by the coordinated release of pancreatic hormones into the bloodstream, particularly insulin and glucagon, and their action on carbohydrate metabolism. Insulin is synthesized and released by the pancreatic β-cell. Therefore, β-cell function is imperative to ascertain physiologic insulin secretion and glucose homeostasis. β-cell dysfunction, that may result in impaired or excessive insulin secretion, plays a critical role in the development of hyperglycaemia and hypoglycaemia.

Diabetes mellitus (DM) and hyperinsulinaemic hypoglycaemia (HH) encompass groups of heterogeneous metabolic disorders that are characterized by hyperglycaemia (DM) or hypoglycaemia (HH). The disorders ultimately manifest as a result of β-cell dysfunction (DM and HH) and β-cell death (DM) leading to defective insulin secretion: insulin secretion is either impaired (DM) or increased and inappropriate for the current blood glucose concentration (HH). The molecular mechanisms and metabolic pathways underlying β-cell dysfunction and β-cell death in DM and HH are not yet fully understood, and the current treatment options for DM and HH are insufficient. Therefore, there is an urgent need to develop new therapeutics.

We characterized the role of pancreatic N-methyl-D-aspartate receptors (NMDARs) in β-cell function and survival and, for the first time, identified aberrant expression of *MCT1* (also known as *SLC16A1),* encoding the monocarboxylate transporter subtype 1 (MCT1), in insulin-producing islet cells of a patient suffering from persistent HH.
Precisely, our data recently published in the journal *Nature Medicine* provide evidence that genetic deletion of pancreatic NMDARs or pharmacologic inhibition of NMDARs increases glucose-stimulated insulin secretion from mouse and human pancreatic islets and improves glucose tolerance in mice and men while leaving basal insulin secretion largely unaffected. In addition, we were able to demonstrate that pharmacologic inhibition of NMDARs enhances islet cell survival under diabetogenic conditions, both in the type 2 diabetic mouse model *db/db in vivo* and in isolated human pancreatic islets *in vitro.*
Our work published in the journal *Diabetologia* reveals that aberrant expression of *MCT1* in human insulin-producing islet cells is associated with exercise-induced hyperinsulinaemic hypoglycaemia (EIHI), a rare form of persistent HH. We report the case of an EIHI patient in which HH was caused by an *MCT1*-expressing insulinoma and identified MCT1 protein in three additional insulinomas.

Taken together, we characterized the role of pancreatic NMDARs in diabetes mellitus and provide evidence that *MCT1* expression in insulin-producing islet cells is associated with EIHI and possibly insulinoma formation. Our findings expand the current knowledge on the pathomechanisms of insulin secretion disorders and may help to develop new therapeutics to maintain normal β-cell function and prevent β-cell death.

Zusammenfassung

Kohlenhydrate sind eine der drei Hauptenergielieferanten des menschlichen Körpers. Insbesondere die ausreichende Versorgung des Gehirns mit Glukose ist essentiell, um eine normale Gehirnleistung gewährleisten zu können. Sowohl zu niedrige Blutzuckerspiegel (Hypoglykämien) als auch erhöhte Blutzuckerspiegel (Hyperglykämien) sind mit akuten lebensbedrohlichen Ereignissen assoziiert (Koma) und können bei wiederholtem Auftreten zu bleibenden Hirnschäden führen (Hypoglykämien) sowie Folgeerkrankungen induzieren (Hyperglykämien). Daher ist es wichtig, den Blutzuckerspiegel innerhalb eines eng definierten Bereichs aufrechtzuerhalten (Glukosehomöostase). Die Glukosehomöostase wird primär über die kontrollierte Freisetzung pankreatischer Hormone in den Blutkreislauf reguliert. Diese werden von den verschiedenen Zelltypen der sogenannten Langerhans-Inseln sekretiert. Insbesondere die Auswirkungen der Hormone Insulin und Glukagon auf den Kohlenhydratstoffwechsel sind für die Aufrechterhaltung eines normalen Blutzuckerspiegels entscheidend. Insulin wird von der pankreatischen Betazelle synthetisiert und freigesetzt. Eine normale Betazellfunktion ist daher eine Grundvoraussetzung, um eine physiologische Insulinsekretion und Glukosehomöostase zu gewährleisten. Dysfunktionen der Betazelle gehen mit einer gestörten Insulinsekretion einher und sind maßgeblich am Auftreten von Hyperglykämien und Hypoglykämien beteiligt.

Die Erkrankung Diabetes mellitus (DM) und das Spektrum Hyperinsulinämischer Hypoglykämien (HH) umfassen eine Gruppe heterogener metabolischer Störungen, die durch das Auftreten von Hyperglykämien (DM) oder Hypoglykämien (HH) charakterisiert sind. DM und HH sind letztlich das Resultat einer bestehenden Betazelldysfunktion (DM und HH) und eines sukzessiven Absterbens der Betazellen (DM). Infolge dessen kommt es zu einer gestörten Insulinsekretion mit einem zunächst relativen, später absoluten Insulinmangel (DM), oder einer für den aktuellen Blutzuckerspiegel inadäquat hohen Insulinsekretion (HH). Die der Betazelldysfunktion und dem Betazelltod zugrunde liegenden molekularen Mechanismen und Signalwege sind bis heute nicht ausreichend verstanden. Die Therapieoptionen zur Behandlung des DM und der persistierenden HH sind unzureichend und die Entwicklung neuer Medikamente ist wünschenswert.

Wir haben herausgefunden, dass pankreatische N-Methyl-D-Aspartat Rezeptoren (NMDAR) die Betazellfunktion und das Überleben der Langerhans-Inseln beeinflussen. Darüber hinaus konnten wir erstmals die Expression des *Monocarboxylat-Transporters 1* (*MCT1,* auch bekannt als *SLC16A1)* in den insulinproduzierenden Inselzellen eines Patienten mit persistierendem HH nachweisen.
So hat unsere Arbeitsgruppe in einer in der Fachzeitschrift *Nature Medicine* veröffentlichten Arbeit zeigen können, dass die genetische Deletion oder pharmakologische Blockade pankreatischer NMDAR die glukose-stimulierte Insulinsekretion muriner und menschlicher Langerhans-Inseln steigert, in verschiedenen Mausmodellen sowie bei Typ 2 Diabetikern die Glukosetoleranz verbessert und unter diabetogenen Bedingungen *in vitro* sowie in einem Mausmodell für den Typ 2 Diabetes mellitus *in vivo* Inselzellschutz vermittelt.
Darüber hinaus konnten wir nachweisen, dass die pathogene Expression von *MCT1* in menschlichen insulinproduzierenden Inselzellen mit einer Sonderform des HH assoziiert ist, bei der es infolge anaerober körperlicher Ausdauerleistungen zu Hypoglykämien kommt (EIHI). In dieser, in der Fachzeitschrift *Diabetologia* veröffentlichten Arbeit, haben wir einen Patienten mit EIHI und Insulinom klinisch charakterisiert und MCT1 in Insulinomzellen nachgewiesen. Zudem haben wir MCT1 in drei weiteren Insulinomen detektiert.

Zusammenfassend haben wir die Rolle pankreatischer NMDAR im Hinblick auf die Erkrankung Diabetes mellitus charakterisiert. Außerdem haben wir zeigen können, dass die Expression von *MCT1* in menschlichen insulinproduzierenden Inselzellen mit EIHI sowie möglicherweise mit der Entstehung von Insulinomen assoziiert ist. Unsere Forschungsarbeiten erweitern den Wissensstand in Bezug auf die Pathogenese von Erkrankungen die mit einer gestörten Insulinsekretion einhergehen. Sie können helfen, neue Medikamente zu entwickeln, die die Betazellfunktion wiederherstellen und den Betazelltod verhindern.

1. Introduction

1.1 The pancreas as a dual-function gland

The pancreas is a lobular organ, located in the upper abdomen in close relation to the stomach, duodenum, and the hilum of the spleen. Encompassing an exocrine and endocrine part, the pancreas is often referred to as a dual-function gland[1].

The exocrine pancreas consists of acinar, centroacinar and duct cells. It synthesizes, stores and finally releases digestive fluids (enzymes, ions and water) into the duodenum of the small intestine. Thus, the exocrine pancreas is a digestive organ, essential for the breakdown and absorption of nutrients[1,2]. The endocrine pancreas contains different types of hormone-secreting cells clustered together in small groups. These cell clusters are scattered among the exocrine tissue and are called the islets of Langerhans named after the German pathologist Paul Langerhans who first described their microscopical appearance in 1869[3,4]. The islets of Langerhans comprise five different hormone-secreting cell types: α-cells that secrete glucagon, β-cells that secrete insulin, δ-cells that secrete somatostatin, pancreatic polypeptide (PP)-cells that secrete pancreatic polypeptide and ε-cells that secrete ghrelin, the latter being important for endocrine pancreas maturation during foetal development but rare in the adult pancreas (less than 1%)[3,5]. Hormones released by the different cell types of the islets of Langerhans play important roles in the process of fuel metabolism and are imperative to maintain blood glucose concentration within a narrow range (glucose homeostasis)[2].

In summary, the pancreas holds a central role in the process of nutrient digestion and absorption (exocrine pancreas) as well as their metabolism and storage (endocrine pancreas), altogether being critical for the control of blood glucose homeostasis[2]. Failure of the exocrine or endocrine pancreas leads to the manifestation of several diseases: whereas exocrine pancreas insufficiency causes malnutrition, dysfunction of the endocrine pancreas primarily affects blood glucose concentration and may result in hypoglycaemia (low blood glucose concentration) or hyperglycaemia (elevated blood glucose concentration)[2,6,7].

1.2 The pancreatic islets

A human pancreas weighs about 80 g and contains approximately 1 million pancreatic islets, equivalent to 1-2% of the organ mass[1,3]. Pancreatic islets are often described as being mini-organs that play a key role in regulating blood glucose concentration by secreting biologically active hormones into the blood stream, particularly insulin and glucagon. The predominant cell types within pancreatic islets are the insulin-secreting β-cells, the glucagon-secreting α-cells and the somatostatin-secreting δ-cells[3]. Non-endocrine cells, e.g. nerve cells that innervate the islet, endothelial cells that constitute the blood vessels and immune cells, such as macrophages and dendritic cells, are also part of an islet[8].

Pancreatic islets are innervated and highly vascularized[8,9]. Although the islets account for only 1-2% of the whole pancreas they receive a disproportionally large fraction of the pancreatic blood supply (up to 5-10%)[3]. Usually an islet is supplied by one to three arterioles that break up into a network of capillaries and exit the islets as venules. A dense capillary network is required to supply the islets with oxygen and nutrients and allows an efficient and fast transport of the islet hormones into the blood stream[8]. Furthermore, in the developing as well as in the adult pancreas, mutual signalling between islet endocrine

cells and endothelial cells has direct implications for pancreas development and islet function: for example, signals from β-cells (e.g. VEGF-A) and vascular-derived signals (e.g. extracellular matrix molecules) have been shown to influence blood vessel formation and islet vascular density as well as β-cell proliferation and function, respectively[8]. Intra-islet interactions of β-cells with endocrine and non-endocrine cells as well as signals derived from extra-pancreatic tissues, e.g. from the gut, liver or adipose tissue further affect β-cell function and proliferation[10].

Immunofluorescence studies of pancreatic islets have revealed substantial interspecies differences with respect to cell type composition, cellular organization and islet innervation[11,12]. For example, human islets contain proportionally fewer β-cells (50-60%) and more α-cells (35-40%) than mouse islets. Furthermore, in rodent islets β-cells are clustered in the core of an islet and are surrounded by α-, δ- and PP-cells. In contrast, in human islets all endocrine cell types are found scattered throughout the islet allowing the majority of cells to be located close to the islet blood vessels and favouring heterologous and homologous contacts between the islet endocrine and non-endocrine cells (**Figure 1**)[11]. Finally, in contrast to mouse islets, parasympathetic innervation of β-cells is sparse in human islets and invading sympathetic neurons seem to preferentially innervate smooth muscle cells of the blood capillaries, located around and deep within the human islet, rather than acting directly on endocrine cells[12].

Functional *in vitro* studies indicate that the cellular organization and innervation pattern of pancreatic islets has direct implications for islet function[11]. For example, in mouse islets oscillations in membrane potential and intracellular calcium in response to high glucose concentrations (11mM) are synchronized throughout the whole islet. Synchronous oscillatory changes in cytosolic free calcium concentration have been suggested to underlie pulsatile insulin secretion[11,13]. In human islets, in which β-cells are found scattered throughout the islet, calcium responses of β-cells to high glucose are not synchronized, suggesting that human β-cells are functionally segregated. Furthermore, whole human pancreatic islets, but not mouse islets, respond with a distinct increase in calcium to low glucose concentrations (1mM). This might be due to the larger fraction of α-cells within human islets, known to respond with an increase in calcium to low glucose concentrations. Thus, α-cells appear to have a stronger impact on the general activity of the human islet compared to rodent islets[11]. Finally, the unique innervation pattern of sympathetic axons in human islets indicates that sympathetic innervation regulates the release of hormones by modulating islet blood flow rather than by acting on the endocrine cells[9,12].

In summary, the vascularization, cellular organization and innervation pattern of the endocrine pancreas directly affects islet function and appears to be essential to orchestrate a coordinated and efficient release of islet hormones into the blood stream, a prerequisite for the tight control of blood glucose homeostasis.

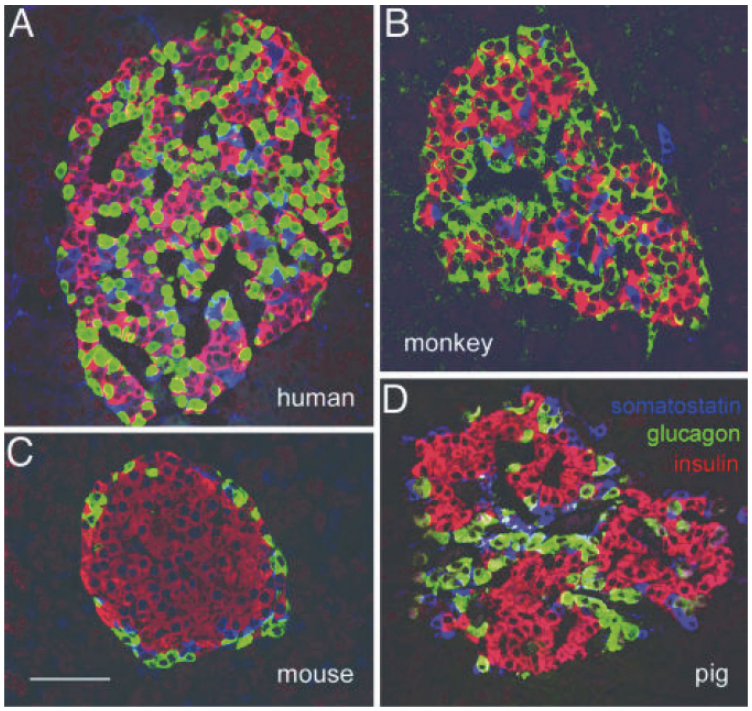

Figure 1: Islets of Langerhans from different species

Over Cabrera et al. Proc Natl Acad Sci USA 2006;103:2334-2339[11]

Representative immunostained pancreatic sections containing islets of Langerhans from human (A), monkey (B), mouse (C), and pig (D). Scale bar 50 µm. In human and monkey islets insulin-positive β-cells (red), glucagon-positive α-cells (green) and somatostatin-positive δ-cells (blue) are found scattered throughout the islet, whereas in mouse islets β-cells are clustered in the core of the islet surrounded by a mantle of α-cells and δ-cells. Pig islets are formed of smaller units of which each appears to have core-mantle organization similar to mouse islets[11].

1.3 Regulation of blood glucose homeostasis

Together with proteins and lipids, carbohydrates are one of the human's three main energy sources. Glucose is required to satisfy quick energy demands and an essential metabolic fuel for normal brain function[14]. Glucose concentration is increased by the ingestion of food followed by its digestion and absorption from the gut and through metabolic pathways that result in the generation of glucose, i.e. hepatic glucose production (gluconeogenesis) and through the breakdown of glycogen stores (glycogenolysis) when energy demands are increased. In contrast its concentration decreases, when glucose is transported from the bloodstream into the cells allowing them to efficiently use glucose as a metabolic fuel[2,14]. Tight regulation of the blood glucose concentration is vital[14]. Both, hypoglycaemia as well as hyperglycaemia can be life-threatening and may induce long-term complications such as permanent brain injury (hypoglycaemia)[15] and cardiovascular disease (hyperglycaemia)[16] when existing over time.

The control of glucose homeostasis is primarily achieved by the coordinated release of pancreatic hormones into the bloodstream, particularly insulin and glucagon, and their action on carbohydrate metabolism (see section 1.5 "Actions of insulin and glucagon")[14]. Other hormones, e.g. adrenalin and

cortisol produced by the adrenal glands contribute to the regulation of blood glucose concentration. These hormones, often referred to as being stress hormones because they are released under stressful conditions, act to raise blood glucose concentration[17,18]. Importantly, insulin, secreted from the pancreatic β-cell, is the body's only hormone capable of lowering blood glucose concentration. Insulin is released following food ingestion, with glucose being the primary nutrient secretagogue[19]. Other fuels, such as amino acids and fatty acids, further increase glucose-induced insulin secretion (GSIS) but generally have only little stimulatory effect on their own[20].

1.4 Mechanisms of glucose-induced insulin secretion (GSIS)

In the β-cell, insulin is stored in secretory granules (SGs). Insulin release, including SGs trafficking to and fusion with the plasma membrane, is a dynamic process that is regulated by various factors including nutrients, circulating hormones, locally released neurotransmitters, and metabolites[19,21].

A prerequisite for GSIS is the uptake and metabolic degradation of glucose by the pancreatic β-cell. Kinetic properties of the proteins involved in these initial steps allow the β-cell to "sense" the current blood glucose concentration and to adjust the amount of insulin required to maintain blood glucose homeostasis: first, low-affinity glucose transporters (GLUTs) act to transport glucose into the β-cell at levels that are proportional to the current blood glucose concentration[2]. In the human β-cell, GLUT1 and GLUT3 appear to be the predominant glucose transporter proteins[22]. Following its uptake, glucose is phosphorylated by glucokinase (GCK), which is an isoform of the enzyme hexokinase. Phosphorylation of glucose by GCK is rate-limiting and therefore GCK is often referred to as being a regulator of glycolytic flux[23]. Hexokinase isoforms differ in their K*m* values, i.e. the substrate concentration at which the reaction is catalysed at half-maximal velocity, and their expression is tissue specific. In the β-cell, GCK has a high K*m* value, meaning that it has a relatively low affinity for glucose, which enables the β-cell to efficiently metabolize and sense a range of glucose without becoming saturated. In contrast, the brain expresses a hexokinase isoform with a lower K*m* value meaning that is has a relatively high affinity for glucose[2]. In addition, the transport of glucose across the blood-brain barrier (BBB) is principally mediated by the insulin-independent facilitative GLUT1[24,25]. This guarantees that the glycolytic needs of the brain are satisfied, even under fasting conditions when glucose and insulin concentrations are low[26].

Following glucose uptake and its phosphorylation, further glucose metabolism in the β-cell mitochondria results in the generation of adenosine-5'-triphosphate (ATP) at the expense of adenosine-5'-monophosphate (ADP). An increase in intracellular ATP/ADP ratio causes ATP-sensitive potassium (K_{ATP}) channels to close, thereby triggering membrane depolarization and opening of voltage-dependent calcium channels (VDCCs)[20]. Calcium influx through VDCCs leads to an oscillatory increase in intracellular Ca^{2+} concentration known to trigger insulin secretion. Calcium mobilization from intracellular stores (e.g. the endoplasmatic reticulum) contributes to the increase in intracellular Ca^{2+}concentration[27]. Thus, the K_{ATP} channel plays a key role in linking cell metabolism to β-cell electrical activity and insulin release[28]. Since it is opened by Mg^{2+}-ADP and closed by ATP, the K_{ATP} channel is often referred to as an energy-sensing channel[29]. The efficacy of calcium on insulin exocytosis is further modulated by K_{ATP} channel independent, so-called amplifying pathways[30].

Both, loss- and gain-of-function mutations of key genes involved in the insulin secretory process are associated with insulin secretion disorders leading to the onset of hypoglycaemia or hyperglycaemia. For example, activating *GCK* mutations and loss-of-function mutations of *ABCC8* and *KCNJ11,* encoding the K_{ATP} channel subunits sulfonylurea receptor 1 (SUR1) and inwardly rectifying potassium channel family 6.2 (Kir6.2) respectively, are known causes of persistent hyperinsulinaemic hypoglycaemia[31,32]. In contrast, loss-of-function mutations within the *GCK* gene and activating K_{ATP} channel mutations can induce monogenic forms of diabetes mellitus, i.e. type 2 Maturity Onset Diabetes of the Young (MODY) and neonatal diabetes, respectively[33,34]. Drugs that either inhibit or open the K_{ATP} channel are well established in the treatment of diabetes mellitus and persistent hyperinsulinaemic hypoglycaemia[35,36].

In the blood, insulin concentration oscillates because insulin is released in a pulsatile fashion[21]. Oscillatory changes of the intracellular calcium concentration are thought to underlie the pulsatile release of insulin[13]. Insulin is thought to be more effective in decreasing blood glucose concentration when it is released in pulses[37]. Accordingly, it has been shown that in type 2 diabetic patients postprandial insulin pulses have a lower amplitude, are less frequent and irregular[21].
Insulin secretion in response to a sustained increase in blood glucose concentration is biphasic: a marked but brief increase in insulin secretion is followed by a decrease to a nadir and a second sustained phase that gradually increases and that lasts as long as glucose is applied[38,39]. The first phase of insulin secretion promotes optimal interstitial insulin concentrations and inhibits hepatic glucose production[40,41]. It depends on a rapid increase in intracellular calcium and is thought to reflect the exocytosis of a pool of readily releasable insulin granules. Loss of the first phase of insulin secretion results in postprandial hyperglycaemia and is an early sign of β-cell dysfunction in type 2 diabetes mellitus[38,42]. The second and sustained phase of insulin secretion requires the continuous elevation of intracellular calcium but also depends on the production of additional not yet fully elucidated signals that amplify the action of calcium on insulin exocytosis[30,38]. It has been suggested that these amplifying pathways may serve to replenish the pool of readily releasable insulin granules either by translocation or granule priming, e.g. by their acidification[38,43]. However, more recent data indicate that three modes of insulin exocytosis exist and that both, the first and the second phase of insulin secretion are caused by granules that are newly recruited and immediately fuse with the plasma membrane[21].

It is generally accepted that the insulin secretory process is further modulated by circulating hormones, locally released neurotransmitters, and metabolites that act either intra- or extracellular to maintain glucose homeostasis[19]. These derive from pancreatic endocrine cells (e.g. somatostatin released by pancreatic δ-cells and acetylcholine released by pancreatic α-cells) or from extrapancreatic tissues (e.g. adrenaline released by the adrenal glands and incretins released by gut cells). Upon activation of somatostatin receptors (SSTRs), somatostatin inhibits insulin secretion by activating K^+ channels of the β-cell plasma membrane leading to K^+ efflux and membrane hyperpolarization. Besides its effect on electrical activity, somatostatin has direct inhibitory effects on insulin exocytosis. Adrenalin binds to α2-adrenoreceptors and exerts similar inhibitory effects on β-cell electrical activity and therefore insulin secretion as somatostatin[19,44]. In contrast, incretins such as glucagon-like peptide-1 (GLP-1) and glucose-

dependent insulinotropic polypeptide (GIP), released by enteroendocrine L and K cells, enhance insulin secretion[45]. Incretins act through 3',5'-cyclic adenosine-monophosphate (cAMP) signalling and activation of downstream molecules including protein kinase A- and exchange protein activated by cAMP (Epac)2A-dependent pathways in response to glucose and other nutrients in the gut lumen. Thus, they play an important role in preventing postprandial hyperglycaemia[46]. Recent data indicate that glutamate, derived from the malate-aspartate shuttle upon glucose stimulation, underlies these stimulatory effects of incretins on insulin secretion[46]. It has long been proposed that glutamate acts as an intracellular mitochondrial-derived messenger that potentiates nutrient-stimulated insulin secretion, being particularly implicated in the second sustained phase of bi-phasic glucose-induced insulin secretion[43,47]. However, the underlying mechanisms remained to be elucidated. Gheni et al. now provide evidence that the uptake of intracellular glutamate into insulin-containing secretory granules by cAMP/PKA signalling potentiates incretin-induced insulin release. It has been suggested that glutamate uptake into SGs may facilitate the recruitment toward and/or fusion of the insulin granules with the plasma membrane[46]. However, several additional biochemical mechanisms how intracellular and extracellular glutamate and its metabolites affect islet function and survival have been proposed. These include extracellular glutamate signalling via α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) and N-methyl-D-aspartate receptors (NMDARs), and the production of metabolic coupling factors upon mitochondrial degradation of glutamate (e.g. ATP, NADPH and fatty acyl-CoA) that enhance GSIS[48].

Besides glutamate, other locally released neurotransmitters modulate insulin secretion in a paracrine or autocrine manner. Acetylcholine (ACh) is released by pancreatic α-cells. Upon binding to its respective receptor (muscarinic ACh receptor M3) on the β-cell plasma membrane, ACh enhances insulin secretion by inducing β-cell membrane depolarization and by inositol triphosphate (IP_3)-induced mobilization of calcium from intracellular stores[19,49]. The neurotransmitter γ-aminobutyric acid (GABA) is released by pancreatic β-cells. Whereas activation of ionotropic GABA receptors on the human β-cell plasma membrane leads to membrane depolarization and therefore enhances insulin secretion, activation of metabotropic GABA receptors has been shown to inhibit insulin secretion[19].

1.5 Actions of insulin and glucagon

Already in 1889, German scientists Oskar Minkowski and Joseph von Mering hypothesized that a pancreatic secrete may be responsible for metabolic control. However, it was not until 1921 that Frederick Banting and Charles Best, under the directorship of John Mcleod from the University of Toronto, started a series of experiments in dogs that finally led to the discovery and isolation of insulin. They demonstrated that saline extracts from the pancreas were able to lower blood glucose concentration in dogs that were rendered diabetic by pancreatectomy. Since its discovery in 1921, the metabolic effects of insulin have been thoroughly studied[26].

Insulin acts as an anabolic hormone: following food ingestion it promotes the cellular uptake and storage of nutrients, precisely glucose, amino acids, and free fatty acids, whereas it inhibits the mobilization of endogenous energy sources. The major target tissues of insulin are the liver, muscle, and adipose tissue[14]. While insulin induces a prompt inhibition of hepatic glucose production (gluconeogenesis), it promotes the synthesis of glycogen (glycogenesis) and suppresses its breakdown (glycogenolysis) in

liver and muscle by stimulating the enzyme glycogen synthetase and inhibiting glycogen phosphorylase. Insulin promotes protein synthesis in the muscle and the *de novo* synthesis of fatty acids (lipogenesis) in liver and adipose tissue, while it suppresses protein and lipid breakdown[14,50].
Insulin passes the BBB and also has profound effects in the central nervous system (CNS), where it regulates energy homeostasis, reproduction, neuronal survival, and high cognition. CNS insulin resistance is associated with Alzheimer's Disease (AD) and depression[51-53].

In contrast, glucagon, released by the pancreatic α-cell when blood glucose concentration decreases, acts to mobilize energy fuels and, in sharp contrast to insulin, increases blood glucose concentration: in brief, glucagon increases hepatic glucose output by stimulating the breakdown of glycogen (glycogenolysis) and by promoting endogenous glucose production (gluconeogenesis). In addition, glucagon has opposing effects on lipid and protein metabolism. Thus, glucagon is an important counterregulatory hormone of insulin[14,54]. The precise mechanisms that act on the α-cell and that promote an efficient release of glucagon even in response to modest changes in blood glucose concentration are not fully understood. Various regulatory signals have been proposed, including inhibitory paracrine signals from neighbouring β- and δ-cells (e.g. insulin and zinc), autocrine signals (glutamate), circulating hormones, and the autonomic nervous system[55,56]. Recently, it has been shown that glucose-induced inhibition of K_{ATP} channels in α-cells suppresses glucagon release by inhibition of voltage-dependent Na^+ channels, leading to reduced action potential height and calcium entry[57].

1.6 Summary

In summary, the homeostatic regulation of blood glucose concentration is complex and requires the coordinated release of hormones into the blood stream. These derive primarily, but not exclusively, from the different cell types of the endocrine pancreas. The cellular organization, rich vascular supply and innervation pattern of the endocrine pancreas facilitates intra-islet interactions and has direct implications for islet function. Extrapancreatic regulatory signals further modify insulin secretion[11,12,19].
The insulin-secreting β-cell holds a central role in maintaining blood glucose homeostasis and β-cell dysfunction that may result in impaired or excessive insulin secretion has been shown to contribute to the development of both, diabetes mellitus and hyperinsulinaemic hypoglycaemia[58-60].

2. Disorders of insulin secretion

2.1 Diabetes mellitus

2.1.1 Introduction

Diabetes mellitus (DM), literally “honey-sweet pass trough” (derived from the Greek word *diabetes* for pass through/siphon and the Latin word *mellitus* for honey-sweet), encompasses a group of heterogeneous metabolic disorders that are characterized by chronic elevation of blood glucose concentration (hyperglycaemia). Hyperglycaemia manifests as a result of defects in insulin secretion, insulin action, or both[61]. Chronic elevation of blood glucose concentration leads to serious long-term complications, primarily affecting the cardiovascular system and subsequently the eyes (retinopathy), kidneys (nephropathy), and nerves (neuropathy)[16]. In brief, the World Health Organization (WHO) projects that diabetes will be the 7th leading cause of death in 2030 and that 50% of individuals with diabetes will die of cardiovascular disease (primarily heart disease and stroke)[62,63]. Furthermore, diabetes is the leading cause of kidney failure and contributes to 1% of global blindness as a consequence of retinopathy[64,65].

Diabetes mellitus has become an epidemic. Globally approximately 350 million individuals have diabetes and both its incidence and prevalence continue to increase[66]. Diabetes mellitus can generally be classified into four different categories: (I) type 1 diabetes mellitus (T1DM), (II) type 2 diabetes mellitus (T2DM), (III) specific types of diabetes mellitus, such as inherited monogenic forms of diabetes, e.g. MODY or neonatal diabetes and (IV) gestational diabetes mellitus (GDM). However, the vast majority of cases fall into the categories (I) and (II)[61].

2.1.2 Type 1 Diabetes mellitus

T1DM, also known as juvenile or insulin-dependent diabetes mellitus, accounts for approximately 5-10% of individuals with diabetes but is one of the most common chronic disorders in children. Its incidence has been increasing in many European countries for several decades, particularly in children younger than 5 years of age[61,67].

T1DM is a typical example of a multifactorial disease: manifestation has a strong hereditary component, with almost 40 genetic loci known to affect disease susceptibility, and is influenced by environmental factors[68]. Many of the loci associated with the risk of T1DM lie within the HLA region on chromosome 6 (*IDDM1* locus) and appear to be involved in immune responses, e.g. the presentation of antigens to the cellular immune system[68]. Environmental factors are poorly defined but may already occur in utero and are thought to contribute to the pathogenesis of T1DM in genetically susceptible individuals[69,70].

Type 1 diabetes mellitus is an autoimmune disorder, characterized by the progressive and selective destruction of pancreatic β-cells by infiltrating autoreactive T-cells and autoantibodies produced by activated B-cells. The characteristic infiltration of pancreatic islets by immune cells (predominantly T-cells, but also B-lymphocytes and macrophages) is termed islet inflammation or insulitis[71]. Since autoimmune β-cell destruction precedes the onset of type 1 diabetes, markers of islet inflammation, i.e. islet-cell autoantibodies, can already be detected in the blood of individuals at risk to develop diabetes[72].

In genetically susceptible individuals circulating autoantibodies against insulin (IAA), glutamic acid decarboxylase (GADA), insulinoma-associated autoantigen 2 (IA2A), and zinc transporter 8 (ZnT8A) can appear as early as 6 months of age with a peak incidence before 2 years of age, meaning that they are detectable months to years before diabetes onset[72]. Importantly, the number of detectable antibody types, and the age at islet autoantibody seroconversion correlates with the risk to develop diabetes: in genetically susceptible children, the 10-year risk to develop type 1 diabetes is 14.5% for those with a single diabetes-related autoantibody (IAA, GADA or IA2A) and 69,7% for those with multiple islet autoantibodies, respectively. The risk of diabetes in children who have no islet autoantibody is 0.4% by the age of 15 years. Furthermore, in children with multiple autoantibodies the progression to diabetes is faster in those who have islet autoantibody seroconversion younger than 3 years of age compared to children 3 years or older (10-year risk 74,9% versus 60.9%)[73]. In other words, not all individuals with islet autoimmunity develop diabetes but the majority of genetically susceptible individuals with multiple autoantibodies progress to type 1 diabetes, and the disease is becoming increasingly predictable. Finally, at disease onset, almost all patients with T1DM have one or multiple antibodies against IAA, GADA, IA2A or ZnT8A. Only 2-4% of patients are autoantibody negative[74].

The rate of β-cell destruction and the extent of β-cell death at disease onset are variable, but it has been estimated that up to 80-95% of β-cells are destroyed when T1DM clinically manifests[61,75]. At the time of diagnosis, individuals with T1DM become generally insulin dependent and they will require a lifelong insulin replacement therapy mimicking physiologic insulin secretion. Even though treatment options have clearly improved over the last decades, management of T1DM remains challenging. Importantly, at the time of diagnosis some β-cells remain functional, and insulin secretion capacity partly recovers in individuals with newly diagnosed T1DM after treatment initiation (honeymoon phase)[76]. In addition, recent studies indicate that residual functional β-cells are present in the majority of type 1 diabetic patients even after 50 years of diabetes, and there is evidence for β-cell regeneration in infants and very young children[77,78]. Therefore, research aims at identifying new treatment strategies to either prevent autoimmune β-cell destruction in patients at risk to develop T1DM (primary and secondary prevention studies), or to halt progressive β-cell destruction and regenerate β-cell function after diabetes onset (tertiary prevention studies). Approaches involve dietary interventions (e.g. the use of extensively hydrolysed infant formula or the timing of gluten introduction)[79,80], antigen-specific therapies (e.g. with insulin)[81], or immune suppression (e.g. with anti-CD20 and anti-CD3 antibodies)[82,83]. However, to date results have been disappointing. None of the numerous T1DM clinical trials could demonstrate permanent effects on β-cell function and exogenous insulin requirement. Even though some immunomodulatory trials led to a transient delay in β-cell destruction and preservation of endogenous insulin secretion, durable and clinically significant improvement of β-cell survival and β-cell function could not be achieved[84].

2.1.3 Type 2 Diabetes mellitus

Type 2 diabetes mellitus accounts for 90-95% of those with diabetes. It is strongly associated with acquired risk factors such as obesity and physical inactivity, but it also has a complex and not yet fully defined genetic component[61,85]. Previously referred to as adult-onset diabetes, T2DM is now becoming more frequent in the paediatric population. Findings from the SEARCH study, assessing changes in the prevalence of T1DM and T2DM among US youths between 2001 and 2009, found an overall increase in type 2 diabetes of 30,5%, which is considerably higher than the overall increase in T1DM prevalence over the same time period (21.1%)[86]. T2DM in the paediatric age group has several unique pathophysiological features: most importantly, deterioration of β-cell function occurs much quicker than in adults[87,88].

T2DM manifests as a result of peripheral insulin resistance, i.e. the diminished tissue response to insulin, and the failure of the β-cell to compensate for peripheral insulin resistance resulting in relative insulin deficiency. Initially, the healthy pancreatic β-cell adapts to changes in insulin action by increasing insulin secretion to maintain normoglycaemia (hyperinsulinaemic phase of T2DM). However, over time β-cell dysfunction develops and β-cell death occurs, leading to a decline in functional β-cell mass. As a consequence hyperglycaemia manifests. Thus, progressive β-cell dysfunction and β-cell death play a critical role in the pathogenesis of type 2 diabetes[85,89]. In fact, postmortem analysis of human pancreata revealed a 40-60% deficit in relative β-cell volume in type 2 diabetic patients that was paralleled by a 3-10-fold increase in β-cell apoptosis[90]. However, others have proposed β-cell dedifferentiation, rather than apoptosis, as the main cause of diabetic β-cell failure[91].

In addition, α-cell dysfunction, that results in inappropriately high glucagon concentrations (hyperglucagonaemia), is now attracting greater attention, since it has been shown to contribute to both, fasting and postprandial hyperglycaemia in type 2 diabetic patients[92].

Insulin resistance and β-cell dysfunction in T2DM are triggered by the release of adipocyte products (lipotoxicity) and increased concentrations of glucose (glucotoxicity)[85]. In obesity, particularly when the mass of visceral and deep subcutaneous adipose depots increases, the secretion of adipokines (peptides that signal the functional status of adipose tissue) is altered: whereas the release of adipokines that impair peripheral insulin sensitivity and/or β-cell function is increased (e.g. leptin), the release of insulin-sensitizing, anti-inflammatory adipokines is decreased (e.g. adiponectin)[85,93]. In addition, adipocyte lipolysis and the release of nonesterified fatty acids (NEFAs) and glycerol are increased in obesity[85,89]. NEFAs induce insulin resistance and impair β-cell function[89,94]. Finally, immune cells infiltrate the adipose tissue and contribute to local and systemic inflammation by increased release of proinflammatory cytokines, e.g. tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and additional mediators of inflammation (e.g. nuclear factor-κB, NF-κB). This further contributes to the development of insulin resistance, β-cell dysfunction and β-cell death[85,89,95].

Although lifestyle appears to play a crucial role in the development of β-cell dysfunction and insulin resistance, several mutations or genetic variants are associated with obesity and insulin insensitivity and therefore pathogenesis of type 2 diabetes. For example, a common variant in the fat mass and obesity-associated (FTO) gene is strongly associated with an increase in body mass index (BMI) and has been identified as obesity-risk allele[96]. Furthermore, variants in the transcription factors hepatocyte nuclear

factor 4 homeobox α (HNF4A), HNF1A, and transcription factor 7-like 2 (TCF7L2) associate with β-cell dysfunction[89] (**Figure 2**).

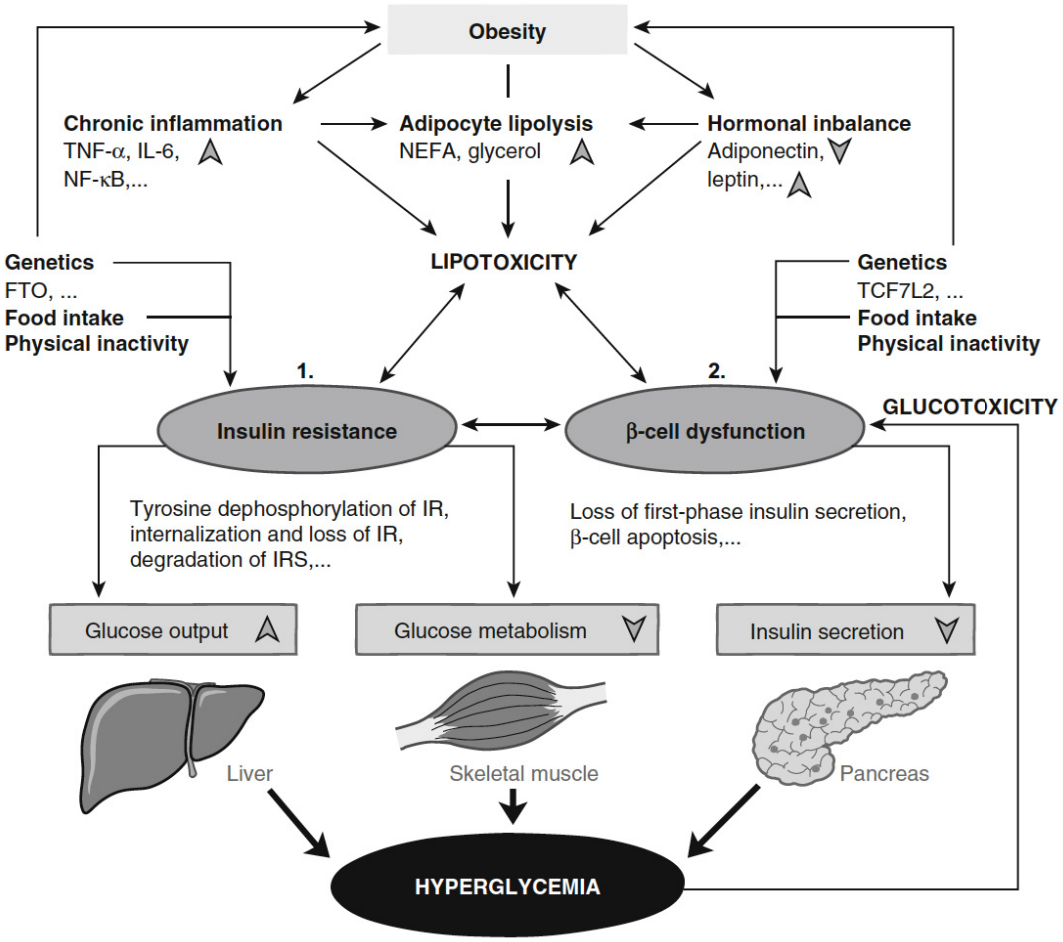

Figure 2: Pathogenesis of type 2 diabetes mellitus

Alena Welters and Eckhard Lammert, Metabolism of Human Diseases 2014;163-169[7]

Type 2 diabetes mellitus manifests as a result of peripheral insulin resistance (1.) and β-cell dysfunction (2.) that develop gradually over time triggered by genetic as well as environmental factors, particularly food intake and physical inactivity. Initially the healthy pancreatic β-cell adapts to changes in insulin action by increasing insulin secretion. However, over time increased concentrations of glucose (glucotoxicity), the increased release of NEFA (lipotoxicity), and an inflammatory response of the adipose tissue contribute to the development of β-cell dysfunction and β-cell death and the compensatory mechanism of the β-cell fails. As a consequence hyperglycaemia manifests. TNF-α: tumor necrosis factor-α, IL-6: interleukin 6, NF-κB: nuclear factor-κB, NEFA: nonesterified fatty acids, FTO: fat mass and obesity-associated gene, TCF7L2: transcription factor 7-like 2, IR: insulin receptor, IRS: IR substrate[7].

T2DM often remains undiagnosed for many years because hyperglycaemia develops gradually and individuals remain asymptomatic for a long time. However, individuals with an increased risk to develop

T2DM, also referred to as having “prediabetes” can be identified by metabolic abnormalities that precede the onset of hyperglycaemia, i.e. impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and increased glycated haemoglobin (HbA_{1C})[61]. IFG, IGT, and increased HbA_{1C} represent an intermediate state of abnormal glucose regulation between normal glucose homeostasis and diabetes, and are predictors for the development of diabetes and cardiovascular disease[97]. With regard to the delay or prevention of diabetes onset, individuals with prediabetes appear to benefit from lifestyle intervention as well as drug therapy (e.g. with metformin or acarbose)[61,97]. Particularly lifestyle intervention that results in physical activity and weight loss seems to be most efficient in preventing diabetes[97].

Consistently, management of T2DM usually starts with lifestyle intervention, including modest exercise and weight reduction. Its success largely depends on the patient’s compliance, and adherence is often difficult to maintain. Therefore, most type 2 diabetic patients require pharmacological intervention to maintain blood glucose concentration within the physiological range. In T1DM, the treatment of choice is the administration of exogenous insulin. In contrast, pharmacological treatment of T2DM first aims at increasing endogenous insulin secretion and enhancing peripheral insulin sensitivity using various glucose-lowering drugs. Several classes of glucose-lowering drugs exist. Biguanides (e.g. metformin) decrease hepatic glucose output and enhances insulin sensitivity, whereas sulfonylureas (e.g. glimepiride) and meglitinides (e.g. repaglinide) directly stimulate insulin secretion by acting on the K_{ATP} channel of the β-cell plasma membrane. Thiazolidinediones (e.g. pioglitazone), also known as insulin sensitizer because they increase insulin sensitivity in muscle and liver, are agonists of the peroxisome-proliferator-activated-receptor-γ (PPAR-γ). Yet another class of drugs delays the absorption of carbohydrates from the gut by inhibiting the enzyme α-glucosidase (e.g. acarbose). The relatively new class of incretin mimetics include GLP-1 receptor agonists (e.g. exenatide) and inhibitor of the GLP-1 degrading protease dipeptidyl peptidase-4 (DPP4 inhibitors, e.g. sitagliptin). Incretin mimetics increase postprandial insulin secretion, suppress glucagon secretion, delay gastric emptying, and reduce appetite[36]. More recently, sodium-glucose co-transporter 2 (SGLT2) inhibitors (e.g. dapagliflozin) have been approved for the treatment of diabetes. These inhibit SGLT2 mediated glucose reabsorption in the proximale convoluted tubule of the kidney, thus facilitating urinary glucose excretion[98,99].

Drug therapy of T2DM typically starts with metformin. However, due to the progressive nature of the disease, most of the type 2 diabetic patients will eventually require a combination of different glucose-lowering drugs and may finally depend on the administration of exogenous insulin. Even though incretin mimetics have been shown to enhance β-cell function, these beneficial effects were either not enough to sustain glycaemic control or lost upon cessation of therapy[100-102]. To date, none of the available antidiabetic drugs can sustainably restore β-cell function and prevent diabetes progression. Furthermore, all glucose-lowering drugs may induce side effects that can be mild (e.g. mild gastrointestinal side effects caused by biguanides and α-glucosidase inhibitors) but also life-threatening (e.g. hypoglycaemia caused by sulfonylureas and insulin)[36,103]. Therefore, there is an urgent need to develop new antidiabetic drugs that maintain blood glucose homeostasis and ideally halt or reverse the progressive decline in functional β-cell mass to prevent disease progression.

2.2 Hyperinsulinaemic hypoglycaemia

2.2.1 Introduction

Hyperinsulinaemic hypoglycaemia (HH) refers to a group of heterogeneous metabolic disorders that are characterized by recurrent episodes of hypoglycaemia. Hypoglycaemia manifests as a result of an unregulated release of insulin from pancreatic β-cells (hyperinsulinism) that is inappropriate for the current blood glucose concentration[104].

HH can be congenital, secondary to perinatal risk factors, e.g. maternal diabetes mellitus or perinatal asphyxia, or may be associated with other metabolic or syndromic disorders, e.g. congenital disorders of glycosylation (CDG) and Beckwith-Wiedemann syndrome, respectively[104]. In adults, the most frequent cause of HH is an insulinoma. Insulinoma are insulin-secreting tumours of pancreatic origin. These tumours are extremely rare (1-4 per million) but the most common endocrine neoplasm of the pancreas[105]. In infancy, the most severe and permanent form of HH is congenital hyperinsulinism (CHI)[35].

2.2.2 Congenital hyperinsulinism

The disorder is rare, affecting approximately 1 in 50.000 newborns in Europe, but is considerably more frequent in inbred populations (1:2.500), and the most common cause of persistent hypoglycaemia in infancy[106]. CHI typically manifests in the newborn period and may cause permanent brain injury: in approximately 60% of all patients with CHI severe hypoglycaemia occurs before 72 h of life. However, late-onset presentation with mildly symptomatic hypoglycaemia in childhood or adolescents may also occur[15,107,108].

Histologically, CHI can be distinguished into two main forms: (I) focal CHI (FCHI), that is defined as an adenomatous hyperplasia of β-cells localized to a single specific location in the pancreas, and (II) diffuse CHI (DCHI) that occurs when all β-cells throughout the entire pancreas are affected[109]. More recently, pancreatic morphology that does not fit into the FCHI or DCHI types has been classified as atypical CHI[110]. Both, clinical and histological variability of CHI are related to the molecular basis of CHI: thus far, mutations in nine different genes have been identified that can be classified into two main categories, i.e. “channelopathies” affecting the K_{ATP} channel and “metabolopathies” affecting different metabolic pathways[60]. However, in as many as 50% of cases the molecular basis of CHI remains unknown suggesting further disease-associated genes[32,106].

2.2.3 Genetic causes of congenital hyperinsulinism

The *ABCC8* and *KCNJ11* genes

The most common causes of CHI are recessive inactivating mutations in the *ABCC8* and *KCNJ11* genes on chromosome 11p15.1 encoding the subunits SUR1 and Kir6.2 of the K_{ATP} channel, respectively. They account for approximately one third of all CHI cases[111]. Thus far, several *ABCC8* and *KCNJ11* mutations have been identified in CHI patients[112]. These either affect the surface expression of the K_{ATP} channel or impair the ability of Mg^{2+}-ADP to stimulate channel activity, both resulting in constant depolarization of the β-cell plasma membrane and therefore increased insulin secretion[31]. Patients with homozygous recessive mutations in the *ABCC8* and *KCNJ11* genes have DCHI[60]. They are often macrosomic at birth and typically present with severe hypoglycaemia in the neonatal period that is often unresponsive to medical

treatment with the K_{ATP} channel opener diazoxide[31,108]. However, recessive *ABCC8* and *KCNJ11* mutations may also lead to the development of FCHI that is the result of a paternally inherited *ABCC8* or *KCNJ11* mutation along with somatic deletion of the corresponding maternal allele within the focal lesion (paternal uniparental disomy of chromosome 11p15.1). Focal CHI accounts for approximately 30-40% of CHI cases[109]. While having a similar clinical presentation, management strategies of FCHI and DCHI differ significantly since FCHI can be cured by surgical removal of the focal lesion[35]. Less frequently single-dominant *ABCC8* mutations can be identified in patients with diffuse CHI. In contrast to recessive mutations, these are mostly associated with a rather mild phenotype and diazoxide responsiveness. In addition, mutation carriers appear to have an increased risk of developing diabetes mellitus[106,113].

Further CHI-associated genes involve *glutamate dehydrogenase* (*GLUD1*), *hepatocyte nuclear factor 4 homeobox* α (*HNF4A*), *HNF1A*, *glucokinase* (*GCK*), *monocarboxylate transporter 1* (*MCT1*), *uncoupling protein 2* (*UCP2*) and *hydroxyacyl-coenzyme A dehydrogenase* (*HADH*) (**Figure 3**). Together, mutations within these genes account for approximately 15% of all CHI cases and are thus much rarer causes of CHI[60].

The *GLUD1* gene

Heterozygous gain-of-function mutations in *GLUD1,* that encodes the enzyme glutamate dehydrogenase (GDH), are the second most common cause of CHI (approximately 6% of CHI cases)[60,106]. Mutations in the *GLUD1* gene are associated with leucine-sensitive hyperinsulinaemic hypoglycaemia[114].

GDH is an intramitochondrial enzyme that plays an important role in regulating amino acid-induced insulin secretion. This enzyme catalyses the oxidative deamination of glutamate to α-ketoglutarate, that enters the tricarboxylic acid (TCA) cycle. Thus, GDH activity promotes ATP synthesis and triggers insulin exocytosis[106]. In the β-cell, the amino acid leucine stimulates insulin release by activating GDH through binding to its catalytic site. In contrast, guanosine-5'-triphosphate (GTP) and ATP inhibit enzyme activity through binding to its allosteric inhibitory site. In CHI, *GLUD1* mutations appear to impair the sensitivity of the enzyme GDH to GTP and therefore result in increased enzyme activity and insulin secretion[106,114].

Patients with *GLUD1* mutations usually have a milder form of CHI that presents during early infancy and is often responsive to diazoxide. Hypoglycaemia typically occurs following the ingestion of a protein-rich meal[32]. In addition, a consistent feature of GDH-CHI is the presence of elevated serum ammonia level (hyperammonaemia) that is thought to arise from renal ammoniagenesis[115].

The *HNF4A* and *HNF1A* genes

Heterozygous loss-of-function mutations in the *HNF4A* gene account for approximately 5% of CHI cases, mutations in the *HNF1A* gene for less than 1% of CHI cases[60]. HNF4A and HNF1A are transcription factors that are expressed in the pancreas and contribute to normal pancreatic β-cell development, growth and function[60]. *HNF4A* and *HNF1A* mutations are both well-established causes of MODY (MODY type 1 and type 3, respectively)[33].

HNF-CHI patients are often macrosomic at birth and are diagnosed with hyperinsulinism within the first week of life[116]. Clinical severity ranges from mild transient hypoglycaemia to persistent hypoglycaemia

that is usually responsive to diazoxide[60,117]. Later in life patients with mutations in the *HNF4A* and *HNF1A* gene appear to switch from hypoglycaemia to hyperglycaemia, and eventually MODY develops, which has been referred to as the "biphasic phenotype". However, the mechanisms by which phenotype switch is caused are still unknown. It has been suggested that early insulin hypersecretion in utero (causing macrosomia) and in the neonatal period contributes to β-cell exhaustion in later life and therefore onset of hyperglycaemia[116,118].

The *GCK* gene

Heterozygous activating mutations in the *GCK* gene, that encodes for the enzyme GCK, account for less than 1% of CHI cases[60]. GCK is often referred to as being a glucose sensor, because its activity is crucial to control glycolytic flux (see section 1.4 "Mechanisms of glucose-induced insulin secretion")[23]. In CHI, activating *GCK* mutations result in an increased affinity of GCK for glucose. As a consequence, the rate of glycolysis is increased, and the threshold for GSIS is reduced leading to an inappropriately high insulin secretion under low glucose conditions[106]. Clinical onset of GCK-CHI varies from onset at birth to adulthood, severity from mildly symptomatic hypoglycaemia to hypoglycaemic seizures. Some mutation carriers might be asymptomatic and both, diazoxide-responsive as well as unresponsive cases have been reported[119,120].

The *HADH* gene

To date, 10 CHI patients have been identified as having an inactivating mutation in the *HADH* gene encoding for the enzyme short-chain L-3-hydroxyacyl-CoA dehydrogenase (SCHAD)[60]. SCHAD is highly expressed in pancreatic β-cells and is known to be crucial for β-oxidation of fatty acids that serve as alternative metabolic fuels during fasting[121]. The exact mechanisms by which *HADH* mutations cause CHI are unknown, but recent data indicate that mutated SCHAD lacks its inhibitory action on GDH, thereby promoting ATP synthesis and increasing insulin secretion upon oxidation of amino acids[122,123]. In addition, SCHAD-CHI patients are severely protein-sensitive, suggesting that under normal conditions SCHAD protects against excessive amino acid-induced insulin secretion[106]. Clinical presentation ranges from severe hypoglycaemia within the first week of life to late-onset hypoglycaemia[60].

CHI-patients with *GLUD1* or *HADH* mutations share several features: both are protein sensitive and, in contrast to patients with *HNF4A*-, or K_{ATP}- channel-mutations, GLUD1- and *S*HADH-CHI typically manifest beyond the neonatal period and patients usually have a normal birth weight[111].

The *MCT1* gene

Heterozygous gain-of-function mutations in the *MCT1* gene (also known as *SLC16A1)*, that encodes for the monocarboxylate transporter subtype 1 (MCT1), cause exercise-induced hyperinsulinism (EIHI). They account for less than 1% of CHI cases[60]. MCT1 is a transmembrane protein responsible for the transport of monocarboxylate metabolites, such as lactate and pyruvate, across the plasma membrane[124]. Under physiological conditions, *MCT1* expression is downregulated in β-cells, preventing entry of extracellular lactate and pyruvate into the cell. The intracellular concentration of lactate and pyruvate is therefore low in β-cells[125]. *MCT1* gene mutations may cause inappropriate transcription of *MCT1* in the pancreatic β-

cell and thus MCT1 protein expression on the cell surface. Consequently, MCT1 allows circulating pyruvate and lactate to enter the β-cell. In the β-cell, pyruvate is metabolized in the TCA cycle resulting in ATP synthesis and ultimately insulin secretion independent of the current blood glucose concentration[126]. Consistently, intravenous application of pyruvate has been shown to increase insulin secretion in 12 patients with EIHI but not in healthy control subjects[127]. As anaerobic exercise results in the accumulation of lactate and pyruvate, MCT1-CHI typically leads to inappropriate insulin secretion following strenuous anaerobic exercise. Patients are usually diazoxide-responsive, but hypoglycaemic episodes may be prevented sufficiently by avoiding anaerobic exercise[60].

The *UCP2* gene

Inactivating *UCP2* mutations have been detected in two patients with unknown causes of CHI[128]. The *UCP2* gene encodes for the mitochondrial carrier uncoupling protein 2 (UCP2) that appears to have proton leak activity, i.e. it transports protons across the inner mitochondrial membrane. Thus, UCP2 separates mitochondrial oxidative metabolism from ATP synthesis and reduces the formation of ATP from glucose. Given the proton leak activity, it has been proposed that UCP2 acts as a negative regulator of insulin secretion[129]. However, data coming from rodent models including *Ucp2*-null mice as well as *Ucp2* transgenic mice revealed conflicting results regarding the effect of *UCP2* expression on insulin secretion, and it is still debated as to whether *UCP2* mutations promote hypoglycaemia[60].

Nevertheless, inactivating *UCP2* mutations have been detected in two CHI-patients that presented with neonatal hypoglycaemia and hypoglycaemic seizures during early infancy, respectively. Both were responsive to diazoxide[128].

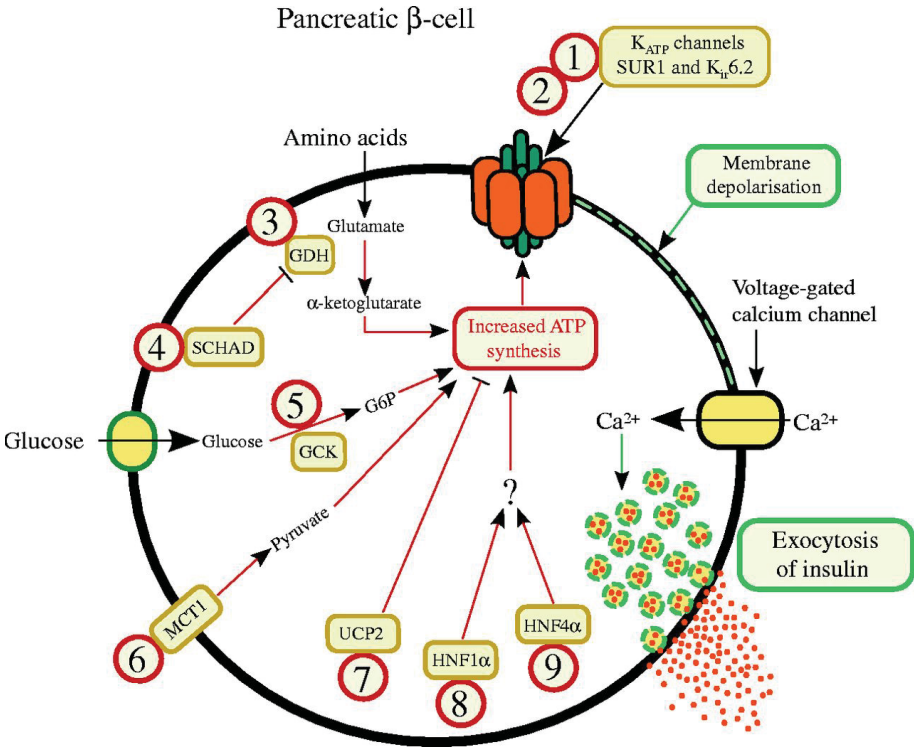

Figure 3: Genetic causes of congenital hyperinsulinism

Sofia A Rahman et al. J Mol Endocrinol 2015;54:R119-R129[60]

To date, mutations in nine different genes have been identified to cause congenital hyperinsulinism. These either affect the functioning of the K_{ATP} channel (1,2) or different metabolic pathways that are involved in the regulation of insulin secretion in the pancreatic β-cell, i.e. the activity of the enzymes GDH (3), SCHAD (4) and GCK (5), cell-surface expression of MCT1 (6) as well as the activity of the mitochondrial carrier protein UCP2 (7) and the transcription factors HNF1A (8) and HNF4A (9)[60].

2.2.4 Treatment of congenital hyperinsulinism

The main goal of CHI treatment is to maintain blood glucose concentration within a physiological range in order to prevent hypoglycaemic brain damage[35]. Initially, this may require the administration of high amounts of glucose (up to 30 g/kg per day), particularly in severe neonatal CHI[108]. Carbohydrates can be provided by frequent and glucose-enriched feeds, but often highly concentrated glucose solutions have to be infused intravenously (up to 15-25 mg/kg per min) which requires the insertion of a central venous catheter[35]. However, in severe CHI this may be insufficient, and additionally continuous or repetitive administration of glucagon (up to 2 mg per day)[108] is needed to prevent hypoglycaemia, either alone or in combination with somatostatin analogues that suppress the release of insulin and GLP-1[130].

Further management of CHI depends on the histological subtype and genetic basis of CHI and includes nutritional, medical and surgical interventions. Whereas focal CHI can be cured by limited resection of the focal area, management of diffuse CHI remains a major challenge[35]. Near-total pancreatectomy in DCHI is associated with high rates of persisting hyperinsulinism (up to 60%), insulin-dependent diabetes

mellitus (nearly 100% after 11 years post-pancreatectomy), and exocrine pancreatic insufficiency (almost 50%)[131]. These data highlight the need for alternative treatment strategies in DCHI, i.e. prolonged medical and nutritional treatment.

First-line drug in the treatment of CHI is the K_{ATP} channel opener diazoxide. By keeping the K_{ATP} channel open, diazoxide reduces β-cell membrane depolarization and thus inhibits insulin secretion[35,132]. However, diazoxide is usually ineffective in patients with recessive inactivating mutations in the *ABCC8* and *KCNJ11* genes that account for many CHI cases[32]. Other treatment options are provided by the long-acting somatostatin analogues octreotide and lanreotide. Compared to the natural hormone somatostatin, these have a prolonged half-life and can therefore be applied by multiple daily subcutaneous (s.c.) injections or continuous s.c. infusion (octreotide), or by a single deep subcutaneous injection every 4 weeks (lanreotide) rather than by continuous intravenous infusion[35,132]. Thereby, discharge from hospital is possible with synthetic somatostatin analogues. Though not approved for this indication, off-label use of somatostatin analogues is common in CHI[133]. However, not all CHI patients respond sufficiently to somatostatin analogues, and their use in the neonatal period has to be assessed carefully because of possible serious adverse effects, particularly necrotizing enterocolitis[134]. Less frequently, antagonists of the voltage-dependent L-type calcium channel, e.g. nifedipine or amlodipine, are used for the treatment of CHI. Since they appear to have only little effect, most hyperinsulinism centres do not consider these drugs as indicated, and they are primarily used in mild CHI or as add-on therapy when diazoxide and/or somatostatin analogues do not sufficiently restore normoglycaemia or following partial pancreatectomy[135-137]. Besides medical treatment, most of the CHI patients additionally depend on a stringent nutritional therapy that involves frequent carbohydrate-enriched meals, the administration of raw cornstarch or protein-restricted diets in particular cases, i.e. GDH- and SCHAD-CHI. In EIHI, patients are advised to avoid anaerobic exercise in order to prevent hypoglycaemic episodes[35,108].

In summary, the outcome of pancreatectomy in DCHI is poor, and many patients with DCHI do not respond sufficiently to medical treatment. Therefore, there is an urgent need for alternative drugs. It has been proposed that the GLP-1 receptor may be a therapeutic target for the treatment of children with CHI. The GLP-1 receptor antagonist exendin-(9-39) suppresses insulin secretion and corrects fasting hypoglycaemia in SUR-1 knockout mice, and a recent pilot clinical study indicated that exendin-(9-39) elevated fasting blood glucose concentrations in nine adolescents/adults that had been treated for CHI during childhood[138]. However, further clinical studies are required to asses the efficacy and safety of the GLP-1 receptor antagonist exendin-(9-39) in congenital hyperinsulinism. More recently, the mammalian target of rapamycin (mTOR) inhibitor sirolimus has been reported to successfully prevent subtotal pancreatectomy in four infants with severe diffuse CHI unresponsive to high doses of diazoxide and octreotide[139]. Still, novel therapeutics need to be developed to prevent recurrent hypoglycaemia and to reduce the risk of permanent brain injury, particularly in those children with diffuse CHI unresponsive to medical treatment[132].

3. Publications

3.1 Characterization of pancreatic NMDA receptors as possible drug targets for diabetes treatment

3.1.1 Summary and scientific context of the published article

Diabetes mellitus affects almost 350 million people worldwide and both, its incidence and prevalence continue to increase in adults and children[66]. β-cell failure is crucial for the progression to diabetes[85,140]. Therefore, interventions that prevent β-cell death and regenerate β-cell function may help to delay, stop or reverse diabetes progression. However, current pharmacological treatment of T2DM primarily acts by increasing endogenous insulin secretion and/or enhancing peripheral insulin sensitivity[36]. Due to the progressive nature of the disease many type 2 diabetic patients eventually require a combination of different glucose-lowering drugs or depend on the administration of exogenous insulin to control blood glucose homeostasis. Diabetes therapy is further complicated by comorbidities (e.g. chronic kidney disease), and serious adverse effects (e.g. hypoglycaemia and lactic acidosis) that can be caused by some of the most commonly prescribed antidiabetic drugs[99]. Therefore, a main goal of diabetes research is the development of novel antidiabetic drugs that improve insulin action, maintain normoglycaemia, assist with weight loss, avoid adverse effects, prevent cardiovascular disease, and halt the decline in functional β-cell mass to stop or reverse diabetes progression[36].

Studies in animal models indicate that the incretin hormone GLP-1 induces β-cell proliferation and β-cell neogenesis, and prevents β-cell apoptosis[141]. However, clinical studies with incretin mimetics show little evidence for a sustained and clinically relevant improvement of β-cell function in diabetic patients[100-102]. Progress has been made in the development of new formulations and administration routes for drug delivery, including once-weekly tablets of DPP4 inhibitors and implantation of miniature osmotic pumps for continuous administration of GLP-1 receptor agonists[99].

In summary, there is no evidence that any of the available antidiabetic drugs exerts clinically significant and durable effects on β-cell function and β-cell mass. The development of a novel β-cell protective pharmacologic agent would also be relevant for individuals with T1DM, the predominant form of diabetes in children and adolescents[72].

Endocrine cells of pancreatic islets and neurons have various receptors and signalling pathways in common, including the release of neurotransmitters, e.g. glutamate[142-144]. We therefore speculated that drugs acting on the CNS may also act on the pancreas and may be useful for the treatment of insulin secretion disorders. In this context, glutamate and its receptors are of particular interest. Glutamate is the major excitatory neurotransmitter in the CNS. However, increased extracellular glutamate concentrations can be toxic to neurons and induce neuronal death, a process referred to as glutamate excitotoxicity[145]. Glutamate-triggered neuronal cell death results from excessive activation of glutamate receptors and N-methyl-D-aspartate receptors (NMDARs) appear to play central role in this process, mainly because of their high calcium permeability[146]. NMDAR are heterotetrameric glutamate-gated cation-permeable ion channels that are widely expressed in the CNS where they are crucial for brain plasticity, neuronal communication and survival. Consistently, NMDAR dysfunction is associated with various neurodegenerative and psychiatric disorders, e.g. AD, stroke and depression. Therefore, research has

focused on the development of NMDA receptor antagonists and on their potential as novel therapeutics for the treatment of these disorders[147].

In contrast, in the pancreas the role of glutamate and NMDARs is largely unexplored and both, *in vitro* and *in vivo* studies investigating the effect of either NMDAR activation or inhibition on insulin secretion or glucose tolerance have been contradictory[148-152]. It has long been proposed that glutamate acts as an intracellular mitochondrial-derived messenger that couples glucose metabolism to insulin secretion and enhances nutrient-stimulated insulin secretion (see section 1.4 "Mechanisms of glucose-induced insulin secretion")[43,46,47]. In fact, pancreatic islets express components necessary for glutamate signalling, e.g. vesicular glutamate transporters to store glutamate in intracellular vesicles and glutamate receptors such as AMPA, kainate, and NMDARs to bind glutamate and transmit the signal[153]. In addition, consistent with findings in the CNS, it has been demonstrated that the clonal β-cell line βTC3 and human islet β-cells are vulnerable to high extracellular concentrations of glutamate, further suggesting that glutamate homeostasis and signalling in the islets is critical for their function and survival[154].

We therefore hypothesized that pancreatic NMDARs are involved in the regulation of insulin secretion, blood glucose homeostasis and islet cell viability.
To test this hypothesis, we genetically silenced the *Grin1* gene (encoding GluN1, the obligatory subunit of NMDARs) in rat INS1E insulinoma cells and deleted *Grin1* in the pancreatic epithelium of mice, respectively. In addition, we inhibited NMDARs pharmacologically in INS1E cells, isolated mouse and human pancreatic islets, in a mouse model of human T2DM (leptin-receptor deficient mice, *db/db*), and in type 2 diabetic patients. For pharmacological inhibition the over-the-counter NMDAR antagonistic drug dextromethorphan (DXM), its metabolite dextrorphan (DXO), or the selective and specific experimental NMDAR antagonist MK-801 were used (the latter was only used for *in vitro* experiments). Here, we provide evidence that NMDARs are required to limit GSIS. Inhibition of pancreatic NMDARs increases glucose-stimulated insulin secretion from rat INS1E insulinoma cells, mouse and human pancreatic islets and improves glucose tolerance in mice and men. Importantly, basal insulin secretion remained largely unaffected upon NMDAR inhibition. In addition, we demonstrate that pharmacological inhibition of NMDARs enhances islet cell survival under diabetogenic conditions, both in the type 2 diabetic mouse model *db/db in vivo* and in isolated mouse and human pancreatic islets *in vitro.* Finally, we studied the underlying molecular mechanism and provide a model of NMDAR-regulated insulin secretion. We propose that NMDARs are part of a negative feedback loop that limits GSIS at high glucose concentration to prevent excessive insulin release[155].
In summary, the main advantages of NMDAR antagonists that make them attractive candidates for adjunct treatment of diabetes are the following: firstly, NMDAR inhibition leaves basal insulin secretion largely unaffected and does not introduce hypoglycaemia; secondly, NMDAR inhibition appears to be beneficial for islet physiology, and NMDAR antagonists may hold the potential to reduce or stop diabetes progression in humans.

3.1.2 Published research article

*Marquard, J., *Otter, S., *Welters, A.*, et al.* Characterization of pancreatic NMDA receptors as possible drug targets for diabetes treatment. *Nature medicine* **21**, 363-372 (2015).

*Authors contributed equally to this work

http://www.ncbi.nlm.nih.gov/pubmed/25774850

3.1.3 Outlook

Here, we provide evidence that pancreatic NMDARs are involved in the regulation of insulin secretion, glucose homeostasis and islet cell survival. Using various approaches, we demonstrate that inhibition of NMDARs increases GSIS from mouse and human pancreatic islets, improves glucose tolerance in mice and men, and promotes islet cell survival under diabetogenic conditions, both *in vitro* an *in vivo*[155].

We also propose a model of NMDAR-regulated insulin secretion that involves the interaction of NMDARs with K^+ channels, precisely K_{ATP} channels and Ca^{2+}-activated K^+ channels of intermediate conductance (SK4 channels). However, the mechanisms of interaction between NMDARs and K^+ channels in the β-cell remained elusive. Direct coupling of NMDARs to Ca^{2+}-activated K^+ channels of high conductance (BK-channels) has been demonstrated in interneurons of the rat olfactory bulb[156]. Authors suggested that in these specific neurons, NMDARs are in close proximity to BK-channels, and that Ca^{2+} influx through NMDARs activates BK-channels within a small spatial domain (Ca^{2+} microdomain)[156]. Whether this is also true in pancreatic β-cells requires further investigation.

It is also in specific neurons (subthalamic nucleus neurons, STN neurons) where it has been proven that Ca^{2+} influx through NMDARs induces K_{ATP} currents. In STN neurons, NMDAR-evoked K_{ATP} currents are Ca^{2+}-dependent, require the activation of nitric oxide (NO)-and cGMP-dependent pathways, and are augmented by activation of 5′-adenosine monophosphate-activated protein kinase (AMPK)[29,157]. In the brain, it is well known that NMDAR stimulation increases NO synthase (NOS) activity and promotes NO synthesis[158,159]. It is also well established that NO facilitates K_{ATP} channel opening[160,161]. Therefore, NO-mediated interaction between NMDARs and K_{ATP} channels might be a more common phenomenon that could also take place in pancreatic β-cells. In fact, rat pancreatic β-cells express the constitutive NOS isoforms NOS1 and NOS3, and glucose stimulation has been shown to increase NO in a Ca^{2+}/calmodulin-dependent manner[162]. Furthermore, high concentrations of NO activate K_{ATP} channels, possibly by reducing ATP production[163], and inhibit glucose-induced Ca^{2+} oscillations and insulin secretion in rat pancreatic β-cells[164]. These data support our hypothesis, that in pancreatic β-cells NO acts as a second messenger that mediates the activation of K_{ATP} channels upon NMDAR stimulation. Whether this is true and whether NO action is potentiated by AMPK activation, as it has been shown in STN neurons[29], requires further investigation.

Given the islet-cell protective properties of NMDAR blockade, NMDAR antagonists may hold the potential to reduce or stop diabetes progression. We conclude that NMDAR antagonists may be useful in the treatment of human type 1 diabetes mellitus, particularly since we could show that NMDAR inhibition protects isolated human pancreatic islets against cytokine-mediated cell death *in vitro*. Inflammation and the release of cytokines are known to play a key role in the pathogenesis of T1DM[165]**.** Dextromethorphan has few adverse effects. It is sold as an over-the-counter medication, and it has been proven to be safe even in paediatric patients, since it is used as a cough suppressant in children[166]. Further preclinical studies are required to investigate the long-term effects of NMDAR inhibition on autoimmune-mediated β-cell destruction and β-cell regeneration in the context of human T1DM. In addition, further studies are needed to uncover the molecular mechanisms underlying the islet cell protective properties of NMDAR blockade. In the CNS, NMDAR signalling is involved in both, cell death as well as cell survival and

plasticity[167]. These opposing effects on neuron survival have been attributed to the subcellular localization and subunit composition of NMDARs. It has been proposed that activation of synaptic NMDARs promotes cell survival, whereas activation of extrasynaptic NMDARs induces cell death ("localization hypothesis")[168,169]. In the CNS, extrasynaptic NMDARs antagonize nuclear signalling to cAMP-response element binding protein (CREB-shutoff), block the induction of target genes (e.g. brain-derived neurotrophic factor, BDNF), cause mitochondrial dysfunction and induce cell death[168]. Extracellular signal-regulated kinase 1/2 (ERK1/2)-dependent phosphorylation of the protein messenger Jacob, and its trafficking to the nucleus, appear to encode the origin of synaptic versus extrasynaptic NMDAR signals. Extrasynaptic-induced translocation of dephosphorylated Jacob to the nucleus induces sustained dephosphorylation and thus inactivation of CREB and is associated with cell death[170]. With respect to their role in cell survival, pancreatic NMDARs may resemble extrasynaptic NMDARs. Further studies are required to determine the role of Jacob and CREB in pancreatic islets and NMDAR signalling. Interestingly, in adult mouse β-cells, CREB mediates the protective effects of GLP-1 on β-cell survival, in part by preventing the induction of the cyclin-dependent kinase inhibitor 1A (Cdkn1a), known to promote β-cell apoptosis in response to various stressors[171]. Recently, the calcium-regulated cytosolic phosphatase calcineurin has been shown to regulate human β-cell survival by transcriptional activation of insulin receptor substrate-2 (IRS-2), a known upstream regulator of the phosphatidylinositol 3-kinase (PI3K)/Akt signalling pathway[172]. Since NMDAR inhibition prolongs the plateau fraction of Ca^{2+} oscillations, it has been suggested, that the islet-cell protective properties of NMDAR inhibition might be mediated through calcineurin-dependent signal transduction, resulting in the activation of the PI3K/Akt pathway, known to inhibit apoptosis and to permit β-cell mass expansion in obesity[173].

Finally, DXM passes the BBB and antagonizes central NMDARs[174]. Central NMDARs are crucial for brain plasticity, neuronal communication and survival[147]. This is why NMDAR antagonists can induce acute neurological side effects, e.g. dizziness and nausea[155], and may also cause long-term neurotoxic effects, particularly since diabetes is a chronic disorder and NMDAR antagonists may be taken over years. Therefore, new NMDAR antagonists with higher potency and increased specificity towards pancreatic NMDARs should be developed.

3.1.4 General information and personal contribution

Name of the Journal	Nature medicine
Impact factor (2014)	27.363
Personal contribution	28%
Author position	equally contributing 1st author

Alena Welters' (AW) contribution in detail

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.

Precisely, Alena Welters performed the experiments for the following figures:
Fig. 1e; Fig. 2c,e,h; Fig. 4b,c; Fig. 6a,b,c,d; Suppl. Fig. 2a,b,c,d,e,f,h,i; Suppl. Fig. 4f; Suppl. Fig. 5a,b,c,d,e; Suppl. Fig. 6e,f,g

Alena Welters contributed to the concept of the following figure:
Suppl. Fig. 8

Alena Welters contributed to the coordination of the collaboration resulting in the following figures:
Fig. 2a,b,i,j; Suppl. Fig. 2g

Alena Welters reproduced the results of the following figures to confirm data and to meet the journal's requirements for statistical analysis:
Fig.1b; Fig. 3e; Fig. 5a,b; Suppl. Fig. 1i

Besides experimental work, Alena Welters contributed to a relevant extent to the preparation of the figures, manuscript, supplement and source data files, and contributed to a relevant extent to the statistical analysis.

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

3.2 Association of exercise-induced hyperinsulinaemic hypoglycaemia with *MCT1*-expressing insulinoma

3.2.1 Summary and scientific context of the published article

Exercise-induced hyperinsulinaemic hypoglycaemia (EIHI) is a clinically distinct subtype of congenital hyperinsulinism that is characterized by recurrent episodes of hypoglycaemia induced by anaerobic exercise (see section 2.2.3 "Genetic causes of congenital hyperinsulinism")[60]. It has first been described in 2001 in two adolescents in whom short-term strenuous exercise resulted in a massive burst of insulin secretion and consequently hypoglycaemia. However, at that time the underlying molecular defect of the phenotype remained unknown[175]. Thereafter, Otonkoski and colleges further characterized 12 EIHI patients from two families and demonstrated that an intravenous bolus of pyruvate led to a massive increase of insulin secretion in EIHI patients but not in healthy control subjects[127]. Since it had been known from *in vitro* studies that under normal conditions exogenous lactate or pyruvate do not induce insulin secretion due to negligible transport of the monocarboxylate metabolites across the β-cell plasma membrane[176], authors suggested that the pathogenic mechanisms of EIHI must involve aberrant transport, signalling or metabolism of pyruvate in the pancreatic β-cell. They proposed that a β-cell specific transport defect, possibly affecting cell-type specific expression of *MCT1*, might allow circulating monocarboxylates to enter the β-cell resulting in pyruvate metabolism, ATP synthesis, and ultimately insulin secretion in EIHI patients[127]. Consistent with this hypothesis the expression of *MCT1* is downregulated in β-cells under physiological conditions, and consequently the intracellular concentration of lactate and pyruvate is low [125]. Thus, the organism is protected from hypoglycaemic actions of pyruvate and lactate during exercise or catabolic states[176]. Linkage analysis and sequencing of the EIHI patients that were previously described by Otonkoski et al.[127], revealed activating mutations within the *MCT1* promoter, which induced marked transcriptional increases of *MCT1* expression in patient's fibroblasts and reporter cell lines, including the clonal murine β-cell line (MIN6)[126]. Still, it remained unproven whether *MCT1* mutations cause aberrant *MCT1* expression in β-cells of EIHI patients. Finally, forced β-cell-specific overexpression of *MCT1* in mice was shown to replicate key features of EIHI[177]. However, until recently, it had not been possible to directly determine MCT1 in β-cells from EIHI patients.

For the first time, we here provide evidence that aberrant expression of *MCT1* in human insulin-producing islet cells is associated with EIHI[178]. We report a 16-year-old patient with typical symptoms suggestive of EHIH. Standardized anaerobic exercise led to an increase in plasma lactate concentration, known to correlate with high plasma pyruvate concentration[127], and resulted in a massive release of insulin and symptomatic hypoglycaemia. Magnetic resonance imaging (MRI) and endoscopic ultrasound revealed an insulinoma that was surgically removed. Thereafter, the metabolic response to anaerobic exercise was normal and no further episodes of hypoglycaemia were reported during 9-month follow-up.

Immunohistochemical analysis revealed MCT1 protein in the patient's insulinoma tissue but not in the patient's normal islets. Consistently, real-time PCR and western blot analysis of the insulinoma tissue confirmed high expression levels of *MCT1* mRNA and MCT1, respectively, whereas expression levels were low in control human pancreatic islets. Interestingly, MCT1 was also detected in three of four insulinomas that were additionally studied.

3.2.2 Published research article

*Marquard, J.,*Welters, A.*, et al.* Association of exercise-induced hyperinsulinaemic hypoglycaemia with MCT1-expressing insulinoma. *Diabetologia* **56**, 31-35 (2013).

*Authors contributed equally to this work

http://www.ncbi.nlm.nih.gov/pubmed/23073708

3.2.3 Outlook

We here demonstrate expression of *MCT1* in the insulinoma tissue of an EIHI patient. We also revealed MCT1 protein in three additional insulinomas. Thereby, we provide evidence that *MCT1* expression in insulin-producing islet cells is associated with EIHI, and possibly contributes to insulinoma formation.

Lack of suppression of insulin secretion by exercise had previously been described in three patients with insulinoma[179]. Meanwhile, a fifth insulinoma patient was reported that presented with chest pain and lightheadedness upon fasting and strenuous physical exercise. Hyperinsulinaemic hypoglycaemia was confirmed during an exercise stress test[180]. In these patients, β-cell-specific *MCT1* expression was not investigated. However, their inability to suppress insulin secretion during exercise indicates the presence of MCT1 in the insulinoma tissue. In insulinoma patients, symptoms typically become evident in the fasting state but may also occur following exercise[105]. Hence, *MCT1* expression might be a common feature of insulinoma and MCTs could be involved in tumorigenesis. Further studies are needed to uncover the role of MCT1 in β-cell proliferation and transformation.

Interestingly, MCTs have been shown to be involved in carcinogenesis[181]. In contrast to benign/differentiated cells, cancer cells widely rely on glycolysis for energy production even in the presence of sufficient oxygen, a phenomenon termed “aerobic glycolysis” or “Warburg effect”. Aerobic glycolysis is a relatively inefficient metabolic pathway to generate ATP, and the advantage it confers to cancer cells is not fully understood[182]. Cancer cells appear to adapt to this disadvantage by increasing glucose uptake. Increased rates of glycolysis have repeatedly been observed in cancer cells, and tumour glycolysis has become an attractive target for therapeutic intervention[183]. Given the high rates of glycolysis, considerable amounts of lactate are produced in cancer cells. In addition, pathways other than glycolysis (e.g. glutaminolysis) contribute to the production of intracellular lactate[184]. In this context, MCTs play a dual role in maintaining tumour metabolism. Firstly, lactate efflux via MCTs allows cancer cells to maintain high rates of glycolysis (hyper-glycolytic phenotype). Secondly, pH regulation by co-transport of protons promotes an acidic microenvironement (acid-resistant phenotype) known to be an essential condition for cancer cell survival. Increased expression of MCTs and MCT1 in particular was found in cancers of several organs, including colon, breast, and prostate cancer[181]. Inhibition of MCTs will directly affect tumour metabolism and cancer cell survival. Therefore, MCTs are becoming an attractive therapeutic target for cancer therapy. In different mouse models of human cancer, the *in vivo* application of the non-specific, small-molecule inhibitor of mammalian lactate transporters α-cyano-4-hydroxycinnamic acid (CHC) retarded tumour growth, rendered cancer cells sensitive to irradiation, impaired tumour invasion, and promoted tumour necrosis[185,186]. The development of inhibitors with increased potency and selectivity towards MCT1 resulted in the generation of AZD3965. AZD3965 has been shown to inhibit tumour growth and to enhance radiosensitivity in small cell lung cancer xenografts[187]. A phase I clinical trial is currently conducted to investigate AZD3965 in patients with advanced solid tumours and lymphomas (NCT01791595).

MCT1 inhibition may also hold the potential to inhibit insulinoma growth and may be useful to maintain normoglycaemia in EIHI, particularly when the whole pancreas is affected (diffuse form) and the disorder cannot be cured by surgical resection. Finally, since our data indicate that *MCT1* expression frequency is high in insulinoma (MCT1 was found in 3 out of 4 insulinomas tested), standardized exercise testing is an

important diagnostic tool in patients with symptoms suggestive for exercise-associated hyperinsulinaemic hypoglycaemia.

3.2.4 General information and personal contribution

Name of the Journal	Diabetologia
Impact factor (2014)	6.671
Personal contribution	30%
Author position	equally contributing 1st author

Alena Welters' (AW) contribution in detail

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.

Precisely, Alena Welters performed the experiments for the following figures:
Fig. 2n,o,p

Alena Welters was the leading coordinator of the collaboration resulting in the following figures:
Fig. 2n,o,p

Alena Welters reproduced the results of the following figures:
Fig.2a-f

In sum, the first authors Alena Welters and Jan Marquard contributed equally to the experimental work of this manuscript with approx. 30% each.

3.3 Metabolism of Human Diseases - Diabetes mellitus

3.3.1 Published book chapter

Alena Welters, E.L. Part VI Pancreas, Diabetes Mellitus in *Metabolism of Human Diseases* (ed. Eckhard Lammert, M.Z.) Springer-Verlag Wien 2014, ISBN: 978-3-7091-0714-0

3.3.2 General information and personal contribution

Book Title	Metabolism of Human Diseases
Title	Part VI Pancreas, Diabetes Mellitus
Year	2014
Editors	Eckhard Lammert, Martin Zeeb
Publisher	Springer-Verlag Wien
Personal contribution	70%
Author position	1st author

Contribution in detail

Alena Welters and Prof. Eckhard Lammert together chose the contents and discussed the structure of this book chapter. Alena Welters performed the literature research, wrote the first draft of the manuscript and designed the concept of the figures. Corrections and finalization of the manuscript were conducted in close exchange with Prof. Eckhard Lammert.

3.4 Erstmanifestation des Diabetes: Wie Defekte der Betazellen die Erkrankung induzieren

3.4.1 Published review article

Welters, A., *et al.* Erstmanifestation des Diabetes: Wie Defekte der Betazellen die Erkrankung induzieren. *Deutsches Ärzteblatt, Supplement: Perspektiven der Diabetologie* 110(46):[12] (2013).

3.4.2 General information and personal contribution

Name of the Journal	Deutsches Ärzteblatt, Supplement: Perspektiven der Diabetologie
Impact factor (2014)	3.6 (Deutsches Ärzteblatt)
Personal contribution	70%
Author position	1st author

Contribution in detail

Alena Welters and Prof. Eckhard Lammert together chose the contents and discussed the structure of this review article. Alena Welters performed the literature research and wrote the first draft of the manuscript. Corrections and finalization of the manuscript were conducted in close exchange with Prof. Eckhard Lammert, PD Dr. Thomas Meissner and Prof. Ertan Mayatepek.

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5. List of Abbreviations

ACh	Acetylcholine
AD	Alzheimer's Disease
ADP	adenosine-5'-monophosphate
AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
AMPK	5′-adenosine monophosphate-activated protein kinase
ATP	adenosine-5'-triphosphate
BBB	blood brain barrier
BDNF	brain-derived neurotrophic factor
BK-channel	Ca^{2+}-activated K^{+} channels of high conductance
BMI	body mass index
cAMP	3',5'-cyclic adenosine-monophosphate
CD	cluster of differentiation
CDG	congenital disorders of glycosylation
Cdkn1a	cyclin-dependent kinase inhibitor 1A
cGMP	3',5'-cyclic guanosine monophosphat
CHI	congenital hyperinsulinism
CNS	central nervous system
CREB	cAMP-response element binding protein
DCHI	diffuse congenital hyperinsulinism
DM	diabetes mellitus
DPP4	dipeptidyl peptidase-4
DXM	dextromethorphan
DXO	dextrorphan
EIHI	exercise-induced hyperinsulinism
Epac	exchange protein activated by cAMP
ERK1/2	extracellular signal-regulated kinase 1/2
FCHI	focal congenital hyperinsulinism
FTO	fat mass and obesity-associated
GABA	γ-aminobutyric acid
GADA	glutamic acid decarboxylase autoantibody
GCK	glucokinase
GDH	glutamate dehydrogenase

GDM	gestational diabetes mellitus
GIP	glucose-dependent insulinotropic polypeptide
GLP-1	glucagon-like peptide 1
GLUD1	glutamate dehydrogenase 1
GLUT	glucose transporter
GSIS	glucose-induced insulin secretion
GTP	guanosine-5'-triphosphate
HADH	hydroxyacyl-coenzyme A dehydrogenase
HbA_{1C}	glycated haemoglobin
HH	hyperinsulinaemic hypoglycaemia
HLA	human leukocyte antigen
HNF1A	hepatocyte nuclear factor 1 homeobox α
HNF4A	hepatocyte nuclear factor 4 homeobox α
IA2A	insulinoma-associated autoantigen 2 autoantibody
IAA	insulin autoantibody
IDDM	insulin-dependent diabetes mellitus
IFG	impaired fasting glucose
IGT	impaired glucose tolerance
IL-6	interleukin-6
IR	insulin receptor
IRS	insulin receptor substrate
IRS-2	insulin receptor substrate-2
K_{ATP}	ATP-sensitive potassium channel
MCT1	monocarboxylate transporter subtype 1
Kir6.2	inwardly rectifying potassium channel, subfamily 6.2
MODY	Maturity Onset Diabetes of the Young
mTOR	mammalian target of rapamycin
NEFA	nonesterified fatty acid
NF-κB	nuclear factor-κB
NMDAR	N-methyl-D-aspartate receptor
NO	nitric oxide
NOS	NO synthase
PI3K	phosphatidylinositol 3-kinase

PKA	protein kinase A
PP	pancreatic polypeptide
PPAR-γ	peroxisome-proliferator-activated-receptor-γ
SCHAD	short-chain L-3-hydroxyacyl-CoA dehydrogenase
SG	secretory granule
SGLT2	sodium-glucose co-transporter 2
SK4-channel	Ca^{2+}-activated K^+ channels of intermediate conductance
SLC16A1	solute carrier family 16, member 1
SSTR	somatostatin receptor
STN	subthalamic nucleus
SUR1	sulfonylurea receptor 1
T1DM	type 1 diabetes mellitus
T2DM	type 2 diabetes mellitus
TCA	tricarboxylic acid
TCF7L2	transcription factor 7-like 2
TNF-α	tumor necrosis factor-α
UCP2	uncoupling protein 2
VDCC	voltage-dependent calcium channel
VEGF-A	vascular endothelial growth factor-A
WHO	World Health Organization
ZnT8A	zinc transporter 8 autoantibody

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7. Declarations

Erklärung

Ich versichere an Eides Statt, dass die Dissertation von mir selbständig, ohne unzulässige fremde Hilfe und ohne Benutzung anderer als der angegebenen Hilfsmittel unter Beachtung der „Grundsätze zur Sicherung guter wissenschaftlicher Praxis an der Heinrich-Heine Universität Düsseldorf“ erstellt worden ist. Die aus fremden Quellen direkt oder indirekt übernommenen Gedanken sind ausnahmslos als solche kenntlich gemacht.

Die vorliegende Arbeit wurde weder in dieser noch in ähnlicher Form bei einer anderen akademischen Institution eingereicht.

Am 05.10.2011 wurde mir nach ordnungsgemäßem Promotionsverfahren von der Medizinischen Fakultät der Rheinisch-Westfälischen Technischen Hochschule Aachen der akademische Grad der Doktorin der Medizin (Dr. med.) verliehen.

Dr. med. Alena Welters
Pulheim, den 10. Februar 2016

Declaration

I hereby declare that I have written the present thesis independently, without assistance from external parties and without the use of other resources than those indicated. The ideas taken directly or indirectly from external sources are duly acknowledged in the text.

The work, either in full or in part, has not been previously submitted to any other academic institution.

I was awarded the academic degree Doctor of Medicine (Dr. med.) by the RWTH Aachen University in October 2011 after having demonstrated my scientific competence in an examination procedure in accordance with the statutes of the University.

Dr. med. Alena Welters
Pulheim, 10th of February 2016